

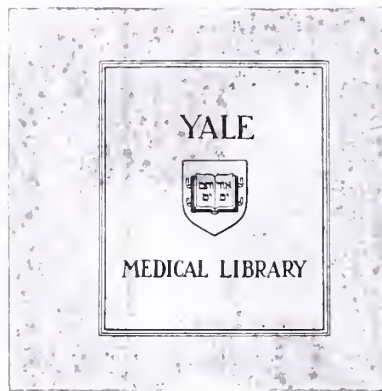


IN VIVO AND IN VITRO STUDIES ON
THE EFFECTS OF ACUTE EXPERIMENTAL ESOPHAGITIS
ON THE FELINE LOWER ESOPHAGEAL SPHINCTER



John Alexander Selling

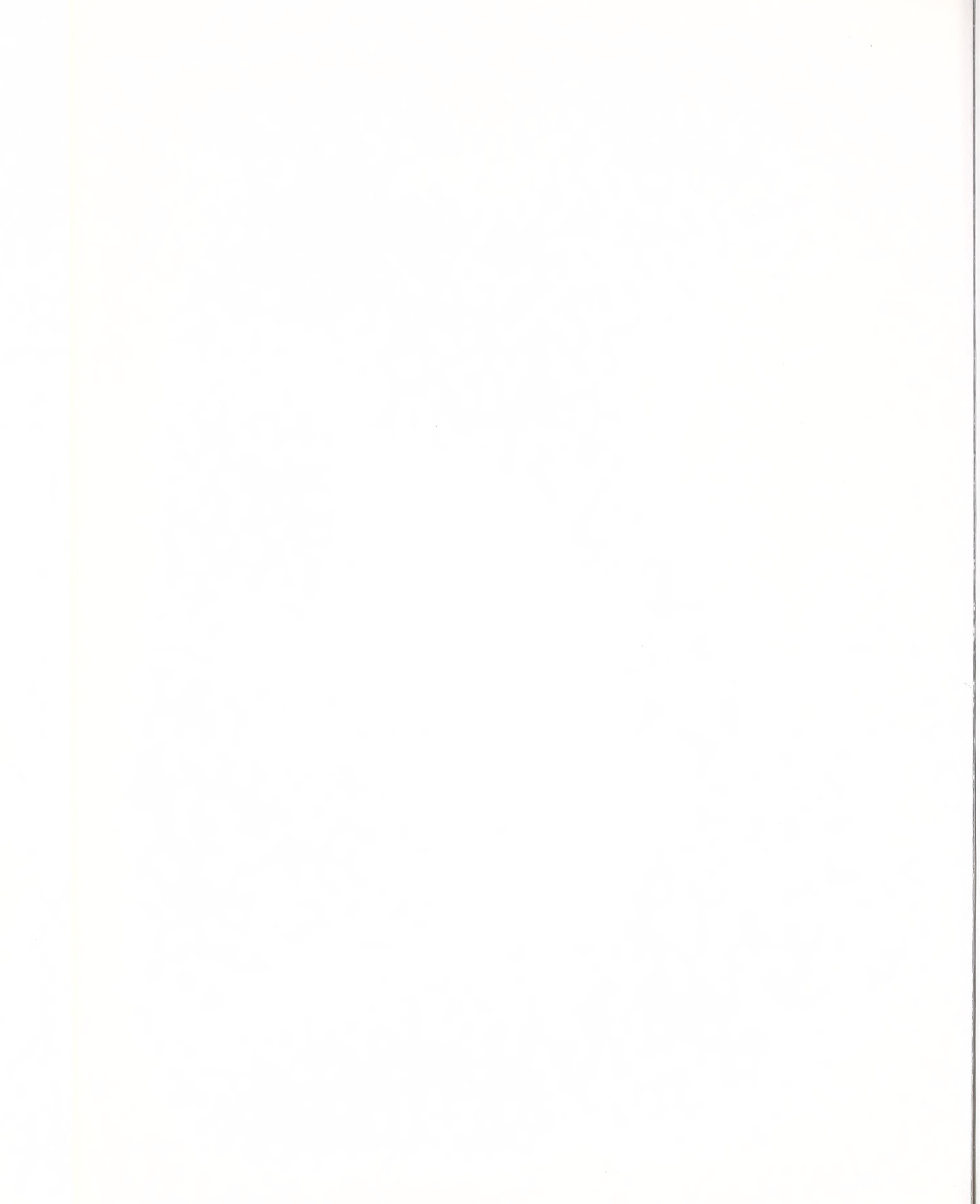
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IN VIVO AND IN VITRO STUDIES ON THE EFFECTS OF ACUTE
EXPERIMENTAL ESOPHAGITIS ON THE FELINE LOWER ESOPHAGEAL SPHINCTER

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A thesis submitted to the Yale University School of Medicine in
partial fulfillment of the requirements for the Degree of Doctor
of Medicine 1980.

THIS THESIS IS DEDICATED TO:

HARRY H. SELLING

CHARLOTTE SELLING

NANCY SELLING

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INTRODUCTION

Reflux esophagitis is a cause of substantial morbidity in the general population. Much effort has been spent in an attempt to elucidate the pathophysiology of gastroesophageal reflux so that a more rational management of this disease process may be developed.

The first portion of this study summarizes the degree to which reflux esophagitis is understood. The normal esophagus is described as well as the changes which occur as a result of reflux esophagitis. The many factors responsible for the development of and/or degree of severity of the resulting esophagitis are then analyzed.

Because the bulk of this study concerns the role of the lower esophageal sphincter in reflux esophagitis, special attention has been directed to explain its function both in the normal subject as well as the patients with esophagitis. The role of various prostaglandins in mediating changes in the lower esophageal sphincter is also discussed.

The second, and most important portion of this work, is devoted to an explanation of the changes that occur in the smooth muscle of the feline lower esophageal sphincter during experimentally induced esophagitis. An animal model of acute esophagitis was developed to yield tissue which could be subjected to different environments in vitro to determine various smooth muscle characteristics. A comparison of the smooth muscle characteristics in various areas of the normal distal esophagus including the lower esophageal sphincter is made. These findings are then contrasted with those found in cats with experimentally induced esophagitis. A discussion of the results follows and various hypotheses involving the role of the lower esophageal sphincter in the production and/or result of reflux esophagitis are proposed.

LITERATURE REVIEW

Histology of the Normal Esophagus

Normally, esophageal mucosa is comprised of a stratified squamous epithelium, a lamina propria, and a muscularis mucosae. The squamous epithelial layer is divided into a basal zone which is responsible for the cellular proliferation of this layer, and an upper stratified zone whose surface lines the lumen of the esophagus.

The lamina propria is comprised of loose vascular connective tissue with lymphocytes, plasma cells, and lymph follicles. Papillae from the lamina propria extend into and between the rete pegs of the squamous layer. Under this lies the muscularis mucosae, separating the mucosa from the submucosa.¹ The muscularis mucosae of the esophagus is the thickest layer of smooth muscle found in the body. The fibers are arranged longitudinally.

The remainder of the esophagus is comprised of three accessory coats; the submucosa, muscularis, and adventitia. The submucosa is a fibro-elastic layer containing 60 to 740 mucus producing esophageal glands, located mainly in the proximal half of the esophagus. The muscularis is composed of an inner circular layer and an outer longitudinal layer. Its composition varies and may be roughly divided into a proximal quarter which consists of skeletal muscle, a middle portion consisting of both skeletal and smooth muscle, and a distal third consisting of regularly arranged smooth muscle. The adventitia is loose and fibrous and connects with surrounding structures, conducting vessels and nerves. Only that portion of the esophagus distal to the diaphragm is covered by a serosa.

Histological Changes in Esophagitis

Various criteria of the changes created by gastroesophageal reflux have been proposed. Those listed below are those of Ismail-Beigi, et al:¹

- 1) Basal cell hyperplasia. The basal zone increases in thickness such that it exceeds its normal 15% of the total thickness of the squamous epithelial layer.
- 2) Elongation and increased vascularity of the papillae of the lamina propria. The papillae extend through more than the normal two-thirds of the thickness of the squamous layer.
- 3) Leucocytic infiltration of both the lamina propria and the squamous layer by neutrophils.¹ Many explanations for these histological changes have been proposed. Livstone, et al were able to suggest an increased cell turnover as the mechanism for basal zone hyperplasia. Their studies show that the amount of (3H)-thymidine uptake by the mucosa is directly proportional to the severity of the changes discussed above.²

All these histological changes are reversible and are secondary to a variety of determining factors which follow. Persistence of the determinants of esophagitis may lead to erosion of the epithelium, ulceration, chronic inflammatory cellular infiltration, fibrosis, and gastric metaplasia. Deep ulceration is followed by irreversible fibrosis. Stricture formation may ensue.

Determining Factors in Reflux Esophagitis

There are many factors responsible for the development of reflux esophagitis. Dodds, et al classifies these determinants of esophagitis with respect to competency of the anti-reflux mechanism, potency and volume of refluxed material, esophageal emptying and tissue resistance.³

Role of LES in Anti-Reflux Mechanism

The lower esophageal sphincter (LES) is probably the most important factor determining competency of the anti-reflux mechanism. "All that is needed for heartburn, please... is lower esophageal sphincter without squeeze."³ The LES is not morphologically identifiable in man, but may be clearly recognized

in other mammals, such as the bat, which spends much time in an inverted position.⁴ In man, the LES is identifiable, however, by manometry, response to various drugs and hormones, and by characteristic force generation in response to stretch in vitro.³ DeCarle in 1975 created gastroesophageal reflux and resulting esophagitis in the Australian bush possum by a sphincteroplasty, temporarily destroying the LES. The LES returned to normal function in 10 weeks with corresponding resolution of the esophagitis.⁵

The role of the LES in prevention of reflux is multifactorial. The LES maintains a resting pressure that is higher than in other areas of the esophagus or stomach. It relaxes during a swallow, permitting passage of a food bolus, and then maintains a sustained contraction, gradually relaxing again to its resting pressure. Patients with symptoms of reflux esophagitis have lower resting LES pressures than normal subjects.⁶ The LES responds to a variety of reflux creating stimuli by increasing tone. Lind, et al showed that an increase in intra-abdominal pressure in man caused an increase in LES pressure greater than that expected due to mechanical impingement.⁷ This LES response to increased intra-abdominal pressure has been found to be defective in patients with reflux esophagitis.³

LES function reflects levels of various enteric hormones as well as cholinergic and other agents. Gastrin had once been implicated as a major determinant of resting LES pressure. When given in pharmacological dosage, gastrin increases LES pressure.⁸ No correlation has been found, however, between basal serum levels of gastrin and LES pressures, either in normal subjects, or in patients with reflux esophagitis.⁶ In addition, other studies have shown that pernicious anemia and the Zollinger-Ellison syndrome are not associated with increased LES pressures although there are very high serum gastrin levels found in these diseases.⁴

Other hormones may also play a role in LES function. The decrease in LES pressure after a fatty meal is most likely secondary to hormonal influence. Secretin, cholecystokinin, and glucagon have all been shown to decrease LES pressure.⁴ Prostaglandins may have some role in regulation of LES pressure and are discussed in detail later. Goyal and Rattan have shown that prostaglandin F2 α increases LES pressure while prostaglandin E2 decreases LES pressure in man.⁹

Neural influences have also been proposed as major determinants of LES pressure. Excitatory vagal cholinergic nerves and alpha adrenergic neurotransmitters have been implicated as increasing LES tone, while relaxation has been elicited by non-cholinergic, non-adrenergic vagal fibers, called "purinergic" nerves as well as by beta adrenergic neurotransmitters.³ Reduction in LES tone in man occurs in certain disorders which damage vagal trunk nerve fibers such as diabetic neuropathy.⁴ Electrical stimulation of the vagus in the opossum produces LES relaxation.¹⁰

The amount of smooth muscle present is a factor in the production of LES pressure. In a study regarding age-related changes of the LES in the rat, Biancani, et al reported that a large increase in the active force generated by the LES occurred between the third and sixth weeks of life.¹¹ They attributed this increase to a slight increase in muscle contractility as well as by a more than threefold increase in the amount of circular muscle present. Further increase in muscle mass with age was not as readily paralleled by increasing LES contractility indicating the existence of other factors such as those being noted.¹¹

In addition to the above factors, there are additional determinants of LES pressure, some of which have been indirectly implied previously. Those additional factors which increase LES tone are decrease in intragastric hydrogen

ion concentration, bethanechol, and metoclopramide. Those additional factors which decrease LES tone are increase in intragastric hydrogen ion concentration, anti-cholinergics, ganglion blockers, nicotine (smoking), ethyl alcohol and chocolate. Surgical procedures which increase LES tone are hiatal hernia repair and fundoplication.^{3,4}

Mechanical Factors Preventing Reflux

Mechanical determinants of LES pressure form another important group in retarding the development of reflux esophagitis. Dodds, et al³ divide these mechanical factors into five groups; valve mechanisms, extrinsic compression, intra-abdominal esophageal segment, mucosal choke, and the spiral stretch mechanism. The valve mechanisms include a mucosal flap, a flutter valve, an acute esophagogastric angle, an abdominal sphincter segment, and the gastric sling fibers. Extrinsic compression at or near the hiatus is created by the diaphragmatic pinchcock, a hepatic tunnel, sling action of the right crus, an esophagogastric "joint", and the phrenoesophageal membrane. The fact that a small portion of the LES lies within the abdominal cavity allows sphincter buttressing by surrounding intra-abdominal pressure. The mucosal choke hypothesis proposes that during sphincter closure, adhesive forces which resist sphincter opening exist between interdigitating mucosal folds. Lastly, Stelzner suggests closure of the terminal esophagus is maintained by a mechanical spiral stretch mechanism.³

It is interesting to note at this point that hiatal hernia disrupts many of these mechanical barriers to reflux, and an increase in esophagitis among persons with hiatal hernia has been repeatedly noted. Although hiatal hernia most likely does predispose to reflux esophagitis, there are many arguments which diminish its role. Behar summarizes these as follows:

- 1) The incidence of sliding hiatal hernia in the general population is 30% to 50%, yet only 5% claim symptoms of reflux esophagitis.
- 2) Endoscopic or radiographic diagnosis of sliding hiatal hernia is often inaccurate leading to bias as an explanation for the association.
- 3) Some hiatal hernias may result from reflux esophagitis, since functional shortening of longitudinal muscle fibers has been induced experimentally with contraction of the esophagus and upward shift of the stomach.
- 4) Reflux symptoms correlate better with sphincter incompetence than with the presence or absence of hiatal hernia.
- 5) Although surgical repair of sliding hiatal hernia leads to symptomatic improvement, it also increases sphincter pressures that could be related to change in length-tension characteristics of the LES circular smooth muscle.⁶

Nature of Refluxed Material

The next group of factors important as determinants of reflux esophagitis concern the nature of the refluxed material and the volume of material available for reflux. Probably the most important substances causing esophagitis are acid and pepsin. Goldberg, et al studies the role of both of these in causing acute esophagitis in the cat. They found that hydrochloric acid alone at pH 1.0 and 1.3 caused esophagitis, while esophagitis did not occur at a pH greater than 1.3 (with one exception). Pepsin, when added to hydrochloric acid at pH 1.0 did not increase the severity of the esophagitis. At pH 1.3, however, the addition of pepsin did increase the severity of esophagitis and at pH's of 1.6 and 2.0, the addition of pepsin created esophagitis. At pH 2.3, the combination of pepsin and hydrochloric acid did not create esophagitis.¹² An explanation of these findings is found when one considers that esophagitis may be caused by different mechanisms. At a pH of 2.0 or less, hydrochloric acid causes esophagitis by denaturation of proteins. Pepsin, on the other hand, becomes maximally active at a pH of 2.0 and causes esophagitis by protein digestion.¹²

In regard to the nature of the material refluxed, substances other than acid and pepsin must be considered in the production of esophagitis. Esophagitis is found frequently among the elderly, many of whom have diminished gastric acid secretion, and has also been found in patients with achlorhydria. Bile has a corrosive effect on the esophageal mucosa and appears to increase the permeability of the esophageal mucosa to hydrogen ion. High concentrations of bile have been found in postprandial samples of gastric contents in patients with reflux esophagitis. Pancreatic enzymes may also have a role in the production of reflux esophagitis.³

Volume of Refluxed Material

Another factor in determining reflux is the volume of the stomach contents and volume of the stomach itself. Increased intragastric pressures would naturally result sooner in patients with subtotal gastrectomies than in patients with normal gastric anatomy if both were given continuous oral feedings. The volume of gastric contents is determined by rate of input versus rate of emptying. The rate of input is a function of eating habitus as well as the gastric secretory rate. The gastric emptying rate is multifactorial. Factors which increase the gastric volume and thus the amount of material available for reflux include obstruction either at the level of the stomach or distally; abnormally slow gastric motility secondary to neural, humoral, inflammatory, or anatomical factors; vagotomy; drugs; and various diseases such as diabetic neuropathy leading to damage to vagal trunk nerve fibers.³

Esophageal Clearance

The rate at which the esophagus clears the refluxed material is important in the production of esophagitis. Many studies have shown that an increase in esophageal mucosal exposure to gastric contents leads to an increase in the rate of development and severity of esophagitis. The esophagus is cleared by primary and secondary peristalsis. Primary peristalsis occurs with swallowing,

creating a contractile wave in the esophagus which propagates distally from the mouth, encouraging esophageal clearing. Various states which discourage swallowing may thus be important in the production of esophagitis. Various theories regarding the development of reflux esophagitis contend that esophagitis probably develops while sleeping when the incidence of swallowing decreases and the horizontal position of the body (especially if supine) creates gravitational assistance in the production of reflux. The rate of swallowing is also influenced by salivation which increases swallowing and thus esophageal clearing, but also increases the volume of gastric material available for reflux.

Alteration of esophageal motility following a swallow has also been implicated in the production of esophagitis. Studies show abnormal esophageal motility in at least 20% of patients with reflux symptoms, although the cause-effect relationship has not been ascertained. The predominant motor abnormality following deglutition appears to be an increased incidence of non-peristaltic and low amplitude contractions, often repetitive, in the distal esophagus which cause ineffective esophageal emptying when the subject is recumbent.³

Secondary peristalsis is induced by esophageal distention such as that created by gastric reflux, resulting in emptying of esophageal content. Diseases such as scleroderma, diffuse esophageal spasm, and severe esophagitis, which decrease the propulsive force or cause motor incoordination, interfere with esophageal clearance, prolonging the duration of contact of refluxed material with esophageal mucosa, and increase the severity of esophagitis.⁶ A similar mechanism may be present in patients with acute post-operative esophagitis in whom esophageal motor function was depressed during anesthesia.³

Mucosal Resistance

Various factors determine the ability of the esophageal mucosa to resist inflammation by gastric contents. Studies have shown that the stratified squamous epithelium lining the esophagus is more sensitive to the digestive

action of gastric juices than any other mucosa in the gastrointestinal tract. Also, the esophagus secretes very little material which can serve as a buffer for refluxed gastric acid. The factors which appear to play a role in decreasing the resistance of esophageal mucosa to refluxed material are as follows:

- 1) Negative nitrogen balance, suggested by animal studies.
- 2) Mucosal changes accompanying aging.
- 3) Alterations in esophageal vascular perfusion, either due to decreased cardiac output or vascular disease.
- 4) Increased frequency of insult.
- 5) The many factors involved in the rate and quality of the healing of esophageal mucosa.³

Another factor which is gaining importance with regards to mucosal resistance is "cytoprotection". Studies in animals indicate that the degree of inflammation created by gastroesophageal reflux may be mediated through prostaglandin formation. Various theories have been proposed. Studies by Brown, et al have suggested that inhibition of prostaglandin synthesis with indomethacin protects the esophagus against development of experimental esophagitis in the cat. In addition, indomethacin prevented the diminished contractility of the LES and the diminished response of the LES to exogenous edrophonium.¹³ Similar studies on animal gastric mucosa suggest other hypotheses. Robert, et al studied the prevention of gastric necrosis in rats and came to the following conclusions:

- 1) Various prostaglandins as well as mild irritants (20% ethanol, 0.35N hydrochloric acid, 0.075N sodium hydroxide, 4% sodium chloride, water at 70°C) are cytoprotective against several necrotizing agents (absolute ethanol, .6N hydrochloric acid, .2N sodium hydroxide, 25% sodium chloride, boiling water) in the stomach.

- 2) Indomethacin blocks the protection afforded by mild irritants, but not by prostaglandin.
- 3) They propose that mild irritants protect the gastric mucosa by inducing the endogenous formation of cytoprotective prostaglandin by the stomach.
- 4) Formation of cytoprotective prostaglandin may be a physiologic phenomenon that maintains the integrity of gastric mucosa cells in spite of the "hostile" nature of the gastric contents.¹⁴

Robert, et al even suggest that cytoprotective prostaglandins may be beneficial in the treatment of reflux esophagitis.¹⁵ It is clear that more work need be done to define the importance of "cytoprotection" and the role of prostaglandins.

Feline LES Smooth Muscle Studies in Esophagitis

The cat, opossum, rat, guinea pig, rabbit, and other animals have been used successfully as experimental models in studies involving the LES and esophagitis. Much of the current work has been done by Castell, et al whose work with feline esophagitis is in part summarized below.

Studies utilizing cats have shown that esophageal inflammation produces LES hypotension.¹⁶ Castell, et al developed a model whereby cats received a 30 minute perfusion of 0.1N HCl, 5 cm above the LES on four consecutive days. This produced biopsy-documented esophagitis and marked decrease in LES pressure which was accompanied by a decreased responsiveness to pentagastrin and also an impaired response to edrophonium. As the cholinesterase inhibitor, edrophonium, requires an intact cholinergic effector unit for its action,¹⁷ these observations indicate that pentagastrin acts at the level of the postganglionic nerve of the cholinergic system.^{18,19} Inflammation may impair the functional integrity of a cholinergic pathway regulating LES tone. LES response to bethanechol, whose action is directly at the neuromuscular junction,¹⁷ was impaired by inflammation. This suggested that LES smooth muscle is unaffected, acutely at least, by inflammation. Morphological studies of the full section

biopsies showed further evidence that the smooth muscle was unaffected by inflammation. Electron microscopy indicated that although neutrophils were observed between muscle bundles in the HCl-perfused cats, the muscle fibers themselves were normal.²⁰

It has been previously noted that patients with symptoms of reflux esophagitis have lower resting LES pressures than normal subjects.⁶ Patients with reflux esophagitis have also been shown to have an impaired response to edrophonium and pentagastrin,^{21,22} as well as a diminished response to bethanechol.^{21,23} Castell, et al explain this disparity between the feline model of esophagitis and patients with reflux esophagitis by pointing out that the feline model deals with acute inflammation, while patients with reflux esophagitis represent a chronic model. They suggest that the chronicity of the esophageal inflammation in reflux patients may have produced smooth muscle dysfunction, so as to impair the LES response to bethanechol. It is further concluded that it seems reasonable to question whether LES smooth muscle dysfunction plays a role in the pathogenesis of gastroesophageal reflux, or is simply a result of chronic inflammation.²⁰

MATERIALS AND METHODS

The present study is comprised of two major components, an in vivo section, and an in vitro section. As much of the methodology is the same, and as many of the cats were used in both studies, they will be described concurrently when possible.

51 adult cats of either sex were selected. They weighed between 3 and 5 kg. Each was anesthetized with ketamine (0.2 cc/kg initially and 0.1 cc/kg \bar{q} 30-45 min. P.R.N.) and had a bite block in position. The in vivo LES was identified with a pressure measuring probe 1.8 mm in outside diameter, having a perfused side opening adjacent to a metal plug obstructing the distal tip. Location of the LES with this one-hole probe was confirmed by at least three pull-through measurements, identifying gastric, LES, and esophageal body pressures with each pull-through. The distance from the LES to the incisors was noted. A "Dent sleeve"⁴³ probe was then positioned with the distal opening registering gastric pressure, the sleeve portion registering LES pressure, and the proximal opening registering esophageal pressure. Distance from incisor to LES was confirmed as were pressure readings from each area with respect to the one-hole measurements. Further verification of LES location was gained from physiologic correlation with pressure tracings by noting the following: 1) Swallowing initially exhibited a rapid very high pressure reading in the esophagus with a simultaneous decrease in pressure or relaxation of the LES. Soon thereafter, the LES pressure would increase rapidly and maintain a sustained after-contraction (Figure 1); 2) the respiratory pattern registered by the esophageal and gastric openings were opposed, being situated on opposite sides of the diaphragm (Figure 2). Having located the LES, with the "Dent sleeve" in place, the catheter was taped to the bite block and the cat allowed to rest until a stable baseline LES pressure was recorded.

Suction biopsies were obtained at 1, 2, 4, and 5 cm proximal to the LES. Samples from the 1 and 5 cm sites were placed in formalin to be reviewed histologically for changes during the course of the experimental period. Samples from the 2 and 4 cm sites were incubated at 37°C for 6 hours with ^3H -thymidine in organ culture. From these latter samples, autoradiographs were prepared, coded, and interpreted blindly by a colleague. For each, 1000 consecutive epithelial cells in the bottom 2 layers of squamous mucosa were examined and ^3H -thymidine labeled cells were counted to determine esophageal mucosal thymidine uptake in response to acute experimental esophagitis. The cats were fasted the night prior to each biopsy, but otherwise allowed to eat normally.

This procedure occurred 3 times during each cat's experimental period: once prior to any acid or drug infusion, once one day following the 4 day acid infusion, and once 21 days following the last day of acid infusion, i.e. day 25 of the experimental period. (This final measurement does not apply to the in vitro cats.) The cats were divided into the following groups.

Acid Control Group

After the initial study on day 1 described above, 17 cats (12 for the in vitro series and 5 in vivo) were administered 0.1N HCl via a polyethylene tube whose distal end was situated 7 cm proximal to the LES. The cat's head and upper body were supported on a foam rubber wedge to promote drainage of the HCl into the stomach. HCl was infused using a Harvard pump at 0.92 cc/min for 30 min on 4 consecutive days. On the 5th and 25th days, LES pressures were recorded and biopsies performed as noted above.

High Dose Indocin (4 mg/kg) Group

15 cats (6 for the in vitro series and 9 in vivo) were treated identically to the acid control group with the exception that they were administered Indocin, 2 mg/kg IV bolus, immediately prior to each HCl infusion and another 2 mg/kg immediately after each HCl infusion.

Low Dose Indocin (150 µg/kg) Group (In Vivo Series Only)

5 cats were treated as above, but with Indocin 75 µg/kg IV bolus immediately prior to each HCl infusion and another 75 µg/kg immediately after each HCl infusion.

Metoclopramide (10 mg/kg) Group (In Vivo Series Only)

5 cats were treated as acid control group, but given metoclopramide, 5 mg/kg IV bolus, immediately prior to, and after each HCl infusion.

Prostaglandin E2 (1 µg/kg/min) Group

5 cats (cats served for both in vivo and in vitro studies) were treated as acid control group, but received prostaglandin E2 at 1 µg/kg/min for 20 min or 18.4 cc at 0.92 cc/min IV infusion via the Harvard pump. The 20 min infusion was completed immediately prior to HCl infusion.

Prostaglandin F2 α (5 µg/kg/min) Group

4 cats (cats served for both in vivo and in vitro studies) were treated as above, but with prostaglandin F2 α at 5 µg/kg/min x 20 min or 18.4 cc at 0.92 cc/min immediately prior to HCl infusion. Note that neither prostaglandin group was biopsied prior to acid infusion, nor were LES pressure measurements made and biopsies achieved on day 25 for these 2 groups. Both did have LES pressures measured prior to and after 4 days of HCl infusion and biopsies were performed on day 5.

Non-acid Control Group

9 cats received no treatment and served as controls for the in vitro series as described below.

In Vitro Studies

36 cats, including 9 non-acid control cats, 12 acid control cats, 6 high dose indocin cats, 5 prostaglandin E2 cats, and 4 prostaglandin F2 α cats were utilized for in vitro studies, after having completed days 1-5 of in vivo studies as described above (the 9 non-acid control cats had no treatment).

On day 5, the cats were anesthetized with ketamine (0.3 cc/kg) and the esophago-gastric junction was exposed. The in vivo LES was again identified with the one-hole probe. With the catheter side opening located at the point of highest pressure, a pin was placed on the outer surface of the LES. The position of the pin was adjusted under fluoroscopy to coincide with the proximal end of the metal plug on the probe. In this manner, the precise location of the side opening was identified on the LES outer surface, and a suture was used to mark the location of the LES high pressure point (Figure 3). Figure 4 shows the esophagus and stomach of a cat in which the LES high pressure point, as well as the upper and lower limits of the ring dissection have been identified.

The stomach, LES, and a 5 cm esophageal segment were removed together and stretched to the previously measured in vivo length. Twenty consecutive transverse rings were cut with razor blades held in a metal block 1.75 mm apart. Twelve adjacent rings were selected from the region of the gastroesophageal junction; the ring identified by the suture, two rings distal, and nine rings proximal. The twelve rings were mounted in a multiple chamber muscle bath as shown in Figure 5. Each ring was suspended between two platinum wire hooks 0.25 mm in diameter. The lower hook was rigidly attached to the bottom of the muscle bath. The upper hook was attached to a force transducer (UC2 cell Statham Instruments) which was mounted on a micrometer stage (Edmund Scientific Co.). The micrometer monitored deformation to within 0.1 mm accuracy. The stretch applied to the rings was measured by the distance between the hooks. After mounting, the rings were allowed to equilibrate for 30 minutes with the distance between the hooks at 12 mm.

Temperature was maintained in the chamber at 37°C by warming the perfusate through a glass coil heat exchanger, heated by water from a constant temperature circulating pump (Haake, model FE), and by regulating the temperature in the water jacket surrounding the muscle chamber. Chamber temperature was monitored with a telethermometer and thermistor probe (Yellow Springs Instrument Co., Inc.).

The bath was perfused continuously with tyrode solution. The tyrode solution contained (in mM/liter): NaCl, 137; NaHCO₃, 12; NaH₂PO₄, 1.8; KCl, 2.7; CaCl₂, 2.7; Glucose, 5.5; and MgCl₂, 1.0. The perfusate was equilibrated with a gas mixture containing 95% O₂ and 5% CO₂ with a pH of 7.2.

After the initial 30 minute equilibration period in tyrode solution, the force developed by each of the rings was recorded (basal force). The solution was then replaced by a tyrode solution to which 140 mM KCl had been added. This solution depolarized the muscle causing a sustained contraction (total force). After 15 minutes, the KCl solution was replaced for 10 minutes by a calcium-free tyrode solution containing 5 mM ethylenediamine tetraacetate disodium salt (EDTA). This solution binds extracellular free calcium causing the muscle to relax completely (passive force). (Figure 6). The force developed by contraction of the muscle (active force) is the difference between total and passive forces. After the muscle was fully relaxed, it was stretched to a new length, and the sequence was repeated. The ring specimens were each tested at 12, 24, 28, 30, 32, 34, 36, 38, and 40 mm distance between the hooks or at least 2 stretches beyond their peak maximal active force generation.

Histological Correlation

After the last stretch and readings, the rings were then placed in individually labeled vials of formalin to be examined histologically for mucosal and muscle damage and for location of the squamocolumnar junction. Both the ring biopsies and the hand suction biopsies were histologically graded in the following manner:

Mucosal Grading

Grade 0: Normal mucosa

Grade 1: Rash (reflux associated squamous hyperplasia)

Grade 2: Esophagitis (as defined earlier) plus inflammation

Grade 3: Esophagitis, inflammation, and mucosal ulceration

Grade 4: Complete absence of, or obliteration of, the definition of the mucosa with granulation tissue

Muscularis Propria Grading

Grade 0: Normal smooth muscle

Grade 1: Invasion by granulation tissue and/or inflammatory tissue with no direct evidence of necrosis or specific muscle fiber involvement

Grade 2: Specific evidence of muscle cell degeneration and necrosis

Dose Response Studies

Each drug used in the previous studies was tested for its pharmacologic effect and to determine effective dosages. Figures 7 and 8 show the change in LES pressure from baseline during administration of the following drugs at the various dosages indicated; Indocin (IV bolus), Metoclopramide (IV bolus), Prostaglandin E2 (IV bolus and 20 min IV infusion), and Prostaglandin F2 α (IV bolus and 20 min IV infusion). Pressures were monitored as previously explained using the "Dent sleeve." The degree of response was measured as the difference between the pressure peak (either positive or negative) developed after drug administration and the resting LES pressure.

RESULTS

Dose Response Studies

As is shown by Figures 7 and 8, prostaglandin E2, prostaglandin F2 α , and metoclopramide increased LES pressure while indocin decreased LES pressure at the dosages tested. While each cat tested absolutely exhibited the above findings, there was significant variability with regards to the degree of response. As the purpose of these dose response tests was to determine effective dosages, the dosages chosen for use in the in vivo and in vitro studies all showed the desired effect in all cats tested. In general, as shown by Figures 7 and 8, increased dosages created increased LES response.

In Vivo Studies

Figure 9 gives the resting LES pressures for the various groups studied on the days tested. Figure 10 displays these results graphically. All groups show a significant ($p < .05$) decrease in resting LES pressure after induction of esophagitis and a return by day 25 of a resting LES pressure similar to that in the basal state. Neither indocin (4 mg/kg), indocin (150 μ g/kg), metoclopramide (10 mg/kg), prostaglandin E2 (1 μ g/kg/min x 20 min), nor prostaglandin F2 α (5 μ g/kg/min x 20 min) had any significant effect in preventing the decrease in resting LES pressure secondary to experimentally induced esophagitis in vivo. Histology study showed that the esophageal mucosa was similar on days 1 and 25 indicating the transient nature of the esophageal inflammation which was present on day 5.

In Vitro Studies

As previously described, the in vitro studies consist of contrasting the normal feline distal esophageal force-length characteristics (non-acid control group) with the force-length characteristics of the non-treated acid control

group, the high dose indocin group, the prostaglandin E2 group, and the prostaglandin F2 α group.

Non-Acid Control Group. Figures 11 through 16 show force-length relationships for a ring corresponding to the high pressure point (LES) as identified by a suture placed under fluoroscopy and for alternative rings taken from the esophageal side, i.e., 2, 4, 6 and 8 proximal, and from the gastric side, i.e., 2 distal. As has been explained, the total force curve was obtained during full muscle contraction in tyrode with KCl, and the passive force curve during relaxation in EDTA. The active force is given by the difference between total and passive forces and represents the force developed by muscle contraction. The basal force is obtained in tyrode solution and the difference between basal and passive force, the basal-active force, represents the level of activation of the muscle while in tyrode solution. Unlike other muscular structures which exhibit no contractile activity while in physiologic solution, muscle from the distal esophagus maintains a state of tonic contracture, which is at least halfway between fully contracted and fully relaxed. In particular, the ring from the high pressure point, which in Figure 12 is designated LES, develops approximately 80% of its full contractile force in tyrode solution. It should be noted, however, that the LES ring is not the only specimen originating from the high pressure zone. Two rings distal and two rings proximal to the LES ring are included in, and form the borders of, the in vivo high pressure zone. As is also shown here, the peak maximal active force is not developed while at the in vivo length, but while stretched to 2 to 4 times the in vivo length.

The same data is presented differently in Figures 17 through 21, where the basal, total, passive, active and basal-active curves of the rings tested are compared to one another. The Figures show that the ring corresponding to

the in vivo high pressure point exhibits the highest basal, total, active, and basal-active curves of all rings tested. The LES passive curve is not significantly different from the rest of the esophageal rings.

Figure 22 shows the maximum active force developed by the LES and esophageal rings in succession. Figure 23 shows the length at which maximum active force (MAF) is developed. The MAF shown in Figure 22 was obtained by averaging the MAF developed by individual rings, regardless of the length at which it occurred. Since all rings did not peak at the same stretched length, averaging active forces at specific lengths, as shown in Figures 11 through 21, yields slightly lower peaks in the active force curves. The MAF developed by the ring corresponding to the in vivo high pressure point is significantly higher than that of adjacent rings. The stretch length of MAF development decreases gradually from the LES region toward the esophagus.

Comparison In Vitro Studies. Figures 24 through 29 display the post-acid companion studies to Figures 11 through 16. These are the force-length relationships for alternate rings 2 distal through 8 proximal taken on day 5 of the experimental period, i.e., after experimental induction of esophagitis. Total, basal, active, passive, and basal-active forces are derived as has been previously described.

The differences in the pre and post-esophagitis studies can be more easily appreciated in Figures 30 through 33. Figure 30 compares the basal forces of adjacent rings in the non-acid control group (labeled control) to those of the acid control group (labeled esophagitis). The ring corresponding to the in vivo high pressure point (HP) is represented by the thick continuous line. In tyrode solution, the basal force of the high pressure point of the non-acid control group is higher than all other adjacent rings. This is in agreement with the findings of Christensen and co-workers.²⁴ In the acid control group, the basal forces of all rings are reduced with respect to the non-acid group. The basal forces of the ring representing the high pressure

point, however, are more profoundly reduced after esophagitis than adjacent rings, such that its forces cannot be distinguished from those basal forces of the other rings, a finding not present in the non-acid control group.

Figure 31 compares the passive forces of adjacent rings in the non-acid control group (labeled control) with those of the acid control group (labeled esophagitis). The passive force curves are similar for both groups. It should be noted that the passive force curve of the high pressure point (solid black line) is not different from those of adjacent rings in either group.

Figure 32 shows the difference between the basal and passive forces (basal-active forces) for the non-acid control group (labeled control) and the acid control group (labeled esophagitis). As expected, the high pressure point ring of the non-acid control group (solid line) has the highest basal-active forces of all rings tested. After the onset of esophagitis, however, the basal-active forces of all rings, especially those of the high pressure point, are essentially eliminated. This finding corresponds directly with the in vivo finding that the LES was not able to develop tonus in basal conditions.

Figure 33 compares the active forces of adjacent rings in the non-acid control group (labeled control) with those of the acid control group (labeled esophagitis). As described, the active force is the difference between the total force, as developed in tyrode with KCl, and the passive force, as developed in tyrode with EDTA. The active force represents the maximum contractile force developed under conditions of maximum stimulation. As shown in the non-acid control group, the ring corresponding to the in vivo high pressure point (solid line) exhibits the highest active forces. In the acid control group, all active forces are reduced, with the active forces of the HP ring being reduced even more prominently than those active forces of adjacent rings, such that it can no longer be distinguished from the others. Also

evident in this Figure is that the maximum active force is developed at approximately 30 mm distance between hooks for both non-acid and acid control groups.

The maximum active forces developed by each individual animal in the non-acid (control) and acid (esophagitis) control groups are compared in Figure 34. For each group, maximum active forces from the high pressure point (HP) represent the LES while the maximum active forces from the esophagus are represented by the fourth ring proximal to the high pressure ring (4P). The mean maximum active force of the LES of normal cats is 12.22 grams. After experimental induction of esophagitis, the mean maximum active force of the LES is reduced to 7.85 grams, a significant reduction ($p < 0.01$). The rest of the esophagus, however, generates virtually the same mean maximal active force both prior to and after esophagitis. The normal mean maximum active force at the fourth proximal ring was 9.86 grams while the mean for the esophagitis group was 8.44 grams. Thus, esophagitis reduces the maximum active force developed by the LES, but not the maximum active force developed by the rest of the esophagus under conditions of maximum in vitro stimulation. The maximum active force developed by the LES after esophagitis is comparable to the maximum active force developed by the rest of the esophagus before and after esophagitis.

In Vitro Histologic Correlation. In order to explain the magnitude of the range of LES maximum active forces found in the cats with esophagitis, this group was divided into those with maximum active forces greater than the mean of 7.85 grams and those with maximum active forces less than 7.85 grams. As has been previously explained, each distal esophageal ring studied in vitro was thereafter examined under the light microscope by a pathologist and numerically graded with respect to degree of histologic mucosal damage. Note was also made of the histological status of the muscularis propria. As previously stated,

the grading scale ranged from no mucosal damage or "0" to complete absence of or obliteration of the definition of the mucosa with granulation tissue or "4". Blind gradings of the LES rings of the 7 cats who were able to develop maximum active forces greater than 7.85 grams showed a mean of 1.83 on the 0-4 mucosal histologic scale. Blind gradings of the LES rings of the 5 cats who developed maximum active forces less than 7.85 grams showed a mean of 3.50 on the 0-4 mucosal scale. This indicates that those cats who could develop the higher maximum active forces (greater than 7.85 grams) had less histologic mucosal damage than those cats who could not develop high maximum active forces (less than 7.85 grams) under conditions of maximum in vitro stimulation. A constant observation of mild muscle edema and vacuolization was present in the muscularis propria of most cats, whether controls or pre-treated. These findings were regarded by the pathologist as secondary to tissue manipulation and stretching.

Figures 35 through 39 compare the various length-tension characteristics previously defined with respect to the LES rings of the non-acid control group (labeled control), the acid control group (labeled esophagitis), those cats in the acid control group with above average maximum active forces (labeled MAF >7.85), and those cats in the acid control group with below average maximum active forces (labeled MAF <7.85). The crosses on each figure represent values significantly different than those of the non-acid control group.

The passive forces of the LES rings achieved by each group at the various stretch lengths indicated, i.e. 12, 24, 28, 30, 32, and 34 mm, are displayed graphically in Figure 35. As described, the passive force is that generated by each LES ring while in tyrode solution with EDTA. As is visualized, there is no significant difference in the amount of passive force generated in any of the acid control groups at any of the stretch lengths tested when compared to the non-acid control group. Thus, before and after esophagitis, passive forces at the level of the in vitro LES remain unchanged.

The basal forces of the LES rings of each group at the various stretch lengths are displayed in Figure 36. The basal force is that generated by the LES ring while in tyrode solution. As is shown, the capacity to produce a basal force has been significantly reduced in all of the acid control groups at almost all stretch lengths tested with respect to the non-acid control group. These basal forces after esophagitis are comparable to the passive forces displayed in Figure 35. Thus, after induction of esophagitis, the in vitro LES lost the capacity to maintain a basal tonus in physiologic solution.

Figure 37 displays the total forces of the LES rings of each group at the various stretch lengths. The total force is the force of contraction generated under conditions of maximum stimulation with tyrode and KCl. Here, both the acid control group and those cats of the acid control group with $MAF < 7.85$ show a significant decrease in the total contractile capacity with respect to the non-acid control group. Those animals of the acid control group with less histologic damage and $MAF > 7.85$ had essentially the same total force production as normal cats. Thus, after induction of esophagitis, the in vitro LES shows a diminished capacity to contract when stimulated maximally. The magnitude of the loss of contractility appears to be directly related to the degree of histological damage.

Figure 38 displays the active forces generated at the LES by each group at the various stretch lengths. The active force is equal to the total force less the passive force. Because the passive forces at the LES were essentially equivalent in the acid and non-acid control groups, the changes noted in the total forces are again noted in the active forces. Again, both the acid control group and those of this group with $MAF < 7.85$ show a significant decrease in active force at the LES with respect to the normal cats. Those cats with slight histological damage showed essentially the same LES active force production as normal cats while those with severe mucosal damage showed a significant decrease in in vitro LES contractility.

The basal-active force is equal to the basal force less the passive force. Figure 39 displays graphically the LES basal-active forces at the various stretch lengths. Again, due to the fact that esophagitis did not affect the passive contractile forces of the in vitro LES, the basal-active force results reflect those of the basal forces in that all the cats of the acid control group showed a significant decrease in basal-active forces. This loss of capacity to produce basal forces in vitro is independent of the degree of histological damage.

Figures 40 through 44 are analagous to Figures 35 through 39 except that the various length tension characteristics of the esophagus, represented by the fourth ring proximal to the high pressure ring, are now being displayed. Again, the non-acid control group is compared with the acid control group as a whole and with those cats of the acid control group with $MAF > 7.85$ and those with $MAF < 7.85$. As before, the crosses on the graphs represent values that vary significantly from the non-acid control group.

Figure 40 shows the passive forces of the 4P rings of each group at the various stretch lengths. Here, as at the LES, there is no significant difference in the passive forces between the non-acid control groups and any of the acid control cats. Thus, both at the LES and distal esophagus, in vitro passive forces are the same with or without esophagitis.

The basal forces of the 4P rings of each group are shown in Figure 41. As with the passive forces, there is no significant difference in the basal forces generated when comparing the non-acid control group with any of the acid control groups. When compared with Figure 36, the differing behavior of the LES with the rest of the esophagus is apparent. After induction of esophagitis, the in vitro LES loses the capacity to maintain a basal force while the rest of the in vitro distal esophagus maintains the same basal force before and after esophagitis. This fact was found to be true even in those cats with relatively little histologic damage.

Figure 42 displays the total forces produced at the various stretch lengths by each group at the 4P level. There is no significant difference between the total force generated by the non-acid control group and that generated by the acid control group as a whole. Those cats with the more severe histological damage, however, did show a significant reduction in the total force generated at the 4P ring with respect to the non-acid control cats. The generation of total force was more significantly reduced at the LES than at the 4P ring after induction of esophagitis (Figure 37). The reduction in total force generated in vitro after induction of esophagitis is directly dependent on the degree of histologic damage at the LES and other esophageal levels.

The active forces of the 4P rings of each group are shown in Figure 43. As with the total forces, there is no significant difference between the active force of the non-acid control group and that of the acid control group as a whole. The cats with more severe histologic damage showed a significant decrease in active force production at the 4P ring. Comparing this figure with Figure 38 shows that esophagitis more significantly reduced the in vitro production of active force at the LES than at other distal esophageal levels. The esophagitis-induced reduction in active force production at both the LES and 4P rings is directly dependent on the severity of the histologic damage.

The basal-active forces generated by the 4P rings are shown in Figure 44. There is a significant reduction in basal-active forces produced by the acid control group when compared to the non-acid control group at the 4P ring. Those cats of the acid control group with little relative mucosal damage showed no significant difference in basal-active forces when compared with the non-acid control group. As shown in Figure 39, after esophagitis, all acid

control cats showed significantly reduced basal-active forces at the LES. At the 4P level, the reduction in basal-active forces was directly related to the degree of histologic damage, while at the LES, the reduction was independent of the degree of damage in this study.

In Vitro Indomethacin Studies. As explained in the Materials and Methods Section, 6 cats were treated identically to the acid control group except that they received indocin 2 mg/kg IV bolus immediately prior to and another 2 mg/kg IV bolus immediately after each HCl infusion. Figure 45 displays the passive, basal, total, active, and basal-active forces produced by the LES at the various stretch lengths tested. These results can be compared with the results on Figures 30 through 33 to determine the influence of indomethacin at 4 mg/kg during the experimental induction of esophagitis. The comparison shows that the 6 cats given indocin displayed no significant difference in the production of passive, basal, total, active, and basal-active forces when compared to the acid control group. This holds true both at the LES and other distal esophageal rings (through 8 rings proximal to the LES). Figure 46 illustrates another presentation of the comparison of the indocin and acid control groups. Thus, in this study, indomethacin administered as described, did not influence the changes noted in in vitro basal and active forces in the acid control group when compared with the non-acid controls. Furthermore, there was no significant histologic difference between the esophageal mucosa of the cats receiving HCl and indocin when compared with the acid control group.

In Vitro Prostaglandin E2 and F2 α Studies. Five cats were treated identically to the acid control group except that they received prostaglandin E2 at 1 μ g/kg/min for the 20 min immediately prior to acid infusion. Four cats were treated identically to the acid control group except that they received prostaglandin F2 α at 5 μ g/kg/min for the 20 min immediately prior to acid infusion.

The forces generated by each at the various stretch lengths are shown in Figures 47 and 48 respectively. Both studies suffer from small sample size. There are no significant differences when comparing any of the defined forces generated by the prostaglandin E2 or F2 α group with those of the acid control group or non-acid control group. A notable trend, however, which can be seen by comparing Figure 47 with Figure 33, is that the in vitro active force developed at the LES by the prostaglandin E2 group approaches the LES active force of the non-acid control group, while the LES active force of the acid control group is comparatively lower. Furthermore, the prostaglandin E2 group showed some tendency to less histologic damage than the acid control group. There was no histologic difference between the prostaglandin F2 α group and the acid control group.

³H-Thymidine Uptake Studies

As explained in the Materials and Methods Section, hand suction esophageal mucosal biopsies were performed 2 and 4 cm proximal to the LES prior to acid infusion, 1 day after completion of acid infusion, and after the 3 week recovery period, i.e., days 1, 5, and 25. These were incubated with ³H-thymidine in organ culture. Autoradiographs were prepared, the bottom 2 layers of squamous mucosa were examined, and labeled cells were counted by a colleague. The labeling index (L.I.), defined as the percentage of labeled cells in those 1000 consecutive epithelial cells examined for each biopsy, was determined for each specimen. Figure 49 shows that mean L.I. \pm SEM for both groups (2 and 4 cm proximal to the LES) on experimental days 1, 5, and 25. At 2 cm proximal to the LES, the mean L.I. was 14.3 ± 1.1 on day 1 and 22.2 ± 1.7 on day 5, a significant difference ($p < .005$). On day 25, the mean L.I. was 6.7 ± 1.0 , also significant ($p < .05$) when compared with day 1. At 4 cm proximal to the LES, the mean L.I. was 14.2 ± 1.1 on day 1 which was significantly less ($p < .001$) than the mean L.I. of 30.3 ± 2.1 on day 5. On day 25, the mean L.I. at 4 cm

proximal was 10.0 ± 1.8 , significantly ($p < .05$) different than on day 1. Also, the mean L.I. at 4 cm proximal to the LES was significantly greater ($p < .05$) than the mean L.I. at 2 cm proximal to the LES on day 5 and on day 25. These results indicate that esophageal mucosal thymidine uptake increases promptly in response to experimentally induced acute esophagitis and returns to normal or subnormal values with healing. They also indicate that the site of the most severe esophagitis, as defined by thymidine uptake, is the site of maximal acid exposure, i.e., the location closest to where the HCl is being infused.

DISCUSSION

There are many contributing factors to the development of gastroesophageal reflux. The purpose of this study has been to first define these factors as they are known today, and then to further elucidate and expound upon the role of esophageal smooth muscle as an important factor. The approach is a multidirectional one, involving both in vivo and in vitro analysis, the role of various pharmacologic agents, as well as mucosal response to acid injury and histologic correlations.

In Vivo Studies

Much of what is known about muscle mechanics is derived from data obtained in studies involving striated muscle. The relative scarcity of data on mechanical properties of smooth muscle may be in part due to technical difficulties encountered while studying it. Unlike striated and cardiac muscle, gastrointestinal sphincter smooth muscle relaxes when electrically stimulated. Also, many smooth muscles exhibit a spontaneous resting tone when placed in physiologic solution. This is particularly true of gastrointestinal sphincters. The in vivo LES maintains an area of increased intraluminal pressure in its resting state. The increase in pressure is secondary to anatomic, pharmacologic, and physiologic factors. After experimental induction of esophagitis with HCl infusion, the resting intraluminal pressure in the LES area is reduced such that it becomes indistinguishable from other areas of the esophagus. Three weeks later, however, the resting LES pressure returns to normal. These changes occur independent of anatomic factors as the anatomy remains unchanged. Histology varies significantly during the experimental period, however, warranting studies of the rate and location of mucosal cellular division.

Pharmacologic factors were manipulated in an attempt to alter the extent of in vivo LES pressure change with esophagitis. Two opposing hypotheses were considered with the assumption that the mucosal inflammation resulting from

HCl infusion may be in part mediated by prostaglandin synthesis. The first proposed that inhibition of prostaglandin synthesis would protect the esophagus against the development of experimental esophagitis. The second proposed that the presence of prostaglandin directly or indirectly resulted in a cytoprotective mechanism maintaining the integrity of the mucosal cells. However, none of the pharmacologic regimens tested (indocin at 4 mg/kg, indocin at 150 μ g/kg, prostaglandin E2 at 1 μ g/kg/min for 20 min, prostaglandin F2 α at 5 μ g/kg/min for 20 min, and metoclopramide at 10 mg/kg) had any significant effect on the development of LES hypotension with experimental esophagitis. Possible explanations include the use of inappropriate dosages of the before-mentioned agents, inappropriate timing of their administration, inappropriate route of administration, relatively small sample size, inaccuracy of original hypotheses, etc.

Thus, to summarize the in vivo studies, it can only be said that this model produced a transient LES hypotension which correlated with the histologic findings of transiently inflamed mucosa. Mechanical factors producing resting LES pressure played no role in hypotension development. Attempts to define hormonal control were unsuccessful. The possibility that there is an intrinsic difference in LES muscle with respect to other esophageal smooth muscle rendering the LES more susceptible to HCl is specifically dealt with in the in vitro studies.

In Vitro Studies

In vitro studies of circular muscle strips from various animal species show some unique features for the strips taken from the gastroesophageal junctional area. They develop much steeper force-length curves with respect to circular muscle from the body of the esophagus, and are more sensitive to the effects of several pharmacologic agents. The anatomic location of the high pressure point is found 2-3 mm proximal to the squamocolumnar junction. The

in vivo high pressure zone extends over an area comprising approximately 5 rings of 1.75 mm width each, the high pressure point corresponding to the middle ring. Thus, the rings at the two distal and two proximal locations may be assumed to represent areas of transition LES and fundus, and LES and esophagus respectively, while the middle 3 rings represent the LES proper.

Defining the mechanical properties of the LES is complicated by the fact that it develops some degree of tonic contraction when placed in tyrode solution. The LES, thus, has the ability both to relax and to contract in response to agents that act on receptors located either directly on the muscle or on intrinsic nerves. This characteristic makes it difficult to determine the maximum contractile strength of the circular muscles of the LES. First, full relaxation, to obtain passive contractile force, was obtained with EDTA in tyrode. Full contraction, the active contractile force, could then be determined with tyrode and KCl. The results of this study indicate that the LES develops not only the highest force in tyrode solution, i.e. the highest basal force, but also the highest total, active, and basal-active forces, when compared with other rings in the distal esophagus. Its passive force, however, is not different from that of other rings.

Thus, with respect to adjacent muscles, the LES shows increased maximal contractile strength as well as increased force in tyrode. This increased basal force is entirely due to active tonic contraction, as the passive forces of the LES are the same as those of muscles from adjacent locations. The small shift present in the passive curves of Figure 19 is consistent with the progressive narrowing of the ring lumens toward more proximal locations. The higher active and total forces of the LES could very simply be explained by an increase in the amount of circular muscle in this area. In fact, recent studies of formalin fixed stomachs taken from human cadavers have shown a distinctive thickening of muscle at the gastroesophageal junction, in an area

that is thought to correspond with the in vivo LES. Thus, within the confines of this study, it is likely that the only intrinsic property of LES circular muscle that is distinctive with respect to adjacent muscles is its ability to develop tonus under basal resting conditions.

The mechanism governing this basal tonus has received much attention, although not many concrete answers have resulted. The literature review and work of J. Fox, et al are summarized in part below. The LES of the North American opossum continuously exerts active tone that can be relaxed by field stimulation of nerves. This relaxation is not mediated by norepinephrine or a purine, and although prostaglandins may modulate this response, they do not appear to mediate it. Like other smooth muscles, LES contractions have been shown to be dependent on calcium. The peak force and maximal velocity of shortening are diminished by reducing the extracellular calcium concentration. LES tone is abolished after eliminating extracellular calcium. The work of J. Fox, et al suggested that the free intracellular calcium concentration responsible for producing tone was very dependent on the extracellular calcium concentration and a continuous membrane flux of calcium. There appear to be calcium-dependent action potentials in many smooth muscles which may result from activation of voltage-dependent conductance channels and allow entrance of extracellular calcium to maintain tone. If these calcium channels are always activated at normal membrane polarization, then this mechanism could account for maintenance of LES resting tone.²⁵

Comparison In Vitro Studies

Having defined the various forces generated by the LES and the differences between the LES and the adjacent esophageal muscle in the normal adult cat, this study compares these findings with those found in cats with esophagitis. Histologic damage was graded numerically to determine whether any changes noted were dependent on the degree of inflammation.

The passive forces generated by all rings tested, i.e. 2 distal through 8 proximal, including the LES, with respect to the normal adult cats, were all roughly equivalent. The slightly higher passive forces generated by each successive proximal ring are due to the fact that each ring decreases in diameter as one moves from cardia to mid-esophagus. This is in accordance with the law of Laplace, where for a cylinder, luminal pressure = wall tension divided by the radius of the cylinder. Thus, for a given tension, i.e. the passive tension, intraluminal pressure increases as the radius decreases. The law of Laplace also holds for those animals with esophagitis. The passive forces of these animals did not differ significantly from those of the normal controls. The passive force generated is thus purely a mechanical phenomenon and is independent of histological damage secondary to acid infusion.

The high basal force generated by the LES has been identified in this study as the only intrinsic property of LES circular muscle that is distinctive with respect to adjacent muscle. After induction of esophagitis, however, the capacity of the LES to maintain a basal tonus which separates it from adjacent muscle is lost. This change was independent of the degree of histologic damage in that even those animals with relatively little inflammation lost the capacity to produce a distinctive basal tone. The adjacent esophageal muscle showed no significant reduction in basal tonus after induction of esophagitis. Thus, mucosal inflammation secondary to HCl infusion abolished this intrinsic property of the LES to produce a high basal tone. It is possible that the inflammation directly or indirectly depletes the supply of extracellular calcium or that hormonal controls regulating the membrane flux of calcium are altered by HCl-induced inflammation.

The term "active force" is used to denote the maximum force of contraction which is developed independently of the passive force. Depending on the stretch length, 80% of the active force at the LES can be basal-active force,

i.e. that force developed in physiologic solution exclusive of the passive force. The other 20% of this contractile force becomes important immediately after a swallow, with increased intra-abdominal pressure, with hormonal influence, etc., as an important barrier to gastroesophageal reflux. After induction of esophagitis, the active force generated by all rings is slightly, although not significantly, reduced except at the LES where the dramatic reduction in active force is significant. Furthermore, the reduction in active force is directly proportional to the degree of histologic mucosal damage for all rings. This fact supports the hypothesis that the increase in active force by the LES with respect to adjacent rings is secondary to an increase in local muscle mass. In the LES, as in adjacent rings, an increase in the severity of the histologic damage leads to an increase in the amount of muscle rendered incapable of maintaining an active force. The deterioration of LES basal force with esophagitis, on the other hand, is independent of the degree of histologic mucosal damage. Further support of this hypothesis is uncovered in the finding that maximal active force development at the LES is also inversely proportional to the degree of mucosal inflammation.

Artifactual changes observed in the muscularis propria were regarded as secondary to muscle bath manipulations. Previous studies by Higgs, et al observed normal light and electron microscopy of the muscularis propria after experimental induction of esophagitis.²⁰ Further studies to resolve any question of loss of muscle integrity will require that full thickness specimens be available for light and electron microscopic evaluation prior to introduction into muscle bath studies.

In Vitro Indomethacin, Prostaglandin E2, and Prostaglandin F_{2α} Studies

In an attempt to define mediators and/or modulators of the inflammatory process of esophagitis, indomethacin and prostaglandins E2 and F_α were administered concurrently along with HCl infusions. Prior to this study, there

had been no published in vitro data where cats with esophagitis were compared to similar cats treated with indomethacin, prostaglandin E2 or prostaglandin F2 α . In vivo data, however, tended to support indomethacin as both maintaining resting LES pressure and decreasing the amount of mucosa damage created by artificial induction of esophagitis. The present study provides in vivo and in vitro evidence that neither indomethacin, prostaglandin E2, nor prostaglandin F2 α have any significant effect on either the degree of histologic damage produced or the change in total, active, basal, and basal-active forces generated after induction of esophagitis. As mentioned in the Discussion of the In Vivo Studies, technical explanations for this outcome include inadequate sample size for the prostaglandin groups, inadequate dosages, improper route of administration, etc. With respect to agent dosage and route of administration, however, each of these agents had been shown to have various effects on GI muscle administered as such at these dosages in other studies. Also, each of these agents was shown to have a local effect on the LES in the dose response studies.

The sample size of the prostaglandin groups are small, but allowing for this, various trends are noted and warrant further investigation. The most obvious of these is that the LES active force of the group pre-treated with prostaglandin E2 is very similar to that of the non-acid control group and impressively higher than that of the acid control group. If one hypothesizes that protection of the mucosa, thereby diminishing inflammation enables the LES smooth muscle to generate stronger active contractions, then one could hypothesize that prostaglandin E2 is protecting the mucosa. E type prostaglandins have been found to inhibit gastric acid secretion when given orally and IV.²⁶ Furthermore, this action has been attributed to a direct inhibitory effect on the parietal cell mass possibly mediated by inhibition of gastrin release.²⁷ These findings are usually the ones proposed as explanations for the anti-ulcer properties of prostaglandin E. However, recently

it has been shown that several prostaglandins, when given at doses much lower than their antisecretory dose, still protect gastric mucosal cells against injury and necrosis produced by strong irritants introduced into the gastric lumen.²⁸ Intestinal "cytoprotection" has also been demonstrated as 16, 16-Dimethyl prostaglandin E2 prevents the severe necrotic lesions of the ileum produced in rats by high doses of prednisolone.²⁹ The mechanism of "cytoprotection" is unknown. Some of the possibilities suggest that prostaglandin may cytoprotect by stimulating mucus secretion, by acting on the cyclic AMP system, by modifying the gastric mucosal circulation, or by strengthening cell membranes. Cytoprotective prostaglandin can be produced endogenously by the stomach.²⁸

More directly applicable to the hypothesis that prostaglandin E2 enables the LES to maintain some capacity for active contraction after esophagitis induction is the fact that prostaglandin actions are sensitive to calcium concentrations and to calcium-magnesium ratios. Prostaglandins may release bound calcium, and/or facilitate calcium fluxes, through the cell wall directly or secondarily through changes in intracellular cyclic AMP.³⁰ The results of this study do not offer definite evidence in support of the theorized cytoprotective properties of prostaglandins. This study does suggest a trend in this direction and that further investigation of this area seems warranted, not in the field of prostaglandin inhibitory agents.

³H-Thymidine Uptake Studies

Patients with reflux esophagitis have been shown to have thickening of the basal zone of the esophageal squamous mucosa, as well as papillary elongation. Accelerated cell turnover, as evidenced by an increase in the uptake of ³H-thymidine, has been held responsible for this. This study, however, further defines these proliferative changes in two manners:

1. Proliferative change has been more clearly defined with respect to time as ^3H -thymidine mucosal uptake returned to normal or subnormal levels after a 3 week period.

2. The mucosal area of maximum acid exposure shows a greater increase in cellular proliferation than adjacent areas with less acid exposure. One may speculate that repeated injury to the surface epithelium increases cell proliferation and migration to the surface to compensate for a greater rate of cell loss from the superficial epithelium. The thickening of the basal zone and the elongated papillae may be the morphological consequences of an expanded proliferative compartment, as well as an increased rate of cellular desquamation.²

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FIGURE 1. With "Dent Sleeve" in place, simultaneous readings of esophageal, LES, and gastric intraluminal pressures are shown. At approximately 1 min into the recording, a swallow occurs exhibiting a rapid contraction and rapid relaxation in the esophagus, an initial relaxation followed by a sustained after-contraction by the LES, and essentially no change in gastric pressure.

FIGURE 2. As in Figure 1, the "Dent Sleeve" is recording simultaneous esophageal, LES, and gastric intraluminal pressures. Two pairs of lines are drawn transecting each pressure recording such that simultaneous pressures from a given time period can be compared. As is shown, pressure increases with inspiration below the diaphragm while it decreases above the diaphragm, and decreases with expiration below the diaphragm while it increases above the diaphragm. A swallow occurs toward the end of the recording.

FIGURE 3. Main drawing shows an open abdomen displaying the stomach and distal esophagus with the one-hole probe in place and a needle placed at the high pressure point. The encircled drawing is a rough schematic of the fluoroscopic image showing the location of needle placement with respect to the probe which is also drawn in detail. A suture is placed at the needle location after fluoroscopy.

FIGURE 4. The esophagus and stomach are shown (with the suture marking the high pressure area) mounted on a wax block and stretched to the measured in vivo length. The multiple razor blade cutting apparatus is displayed along with dotted lines showing where the cuts are made.

FIGURE 5. One of the 12 muscle baths which were operating simultaneously is displayed with an esophageal ring specimen stretched between the lower fixed hook and the upper hook which is attached to a force transducer. The transducer was moved up to increase stretch length. Not shown are a separate water jacket for temperature control and the mechanisms for filling and evacuating the chamber.

FIGURE 6. The basic in vitro study is shown. The force generated by the ring is registered continually as it is bathed in tyrode, tyrode with KCl, and calcium-free tyrode with EDTA. After this, the ring is stretched and the cycle repeated.

FIGURE 7 & 8. The change in in vivo LES pressure from its resting state is shown after administration of the noted drugs at the doses indicated.

FIGURE 9. This is a summary of the in vivo data, showing the mean resting LES pressures of each group at days 1, 5, and 25 of the experimental period. The p values are given comparing the pre and post-esophagitis groups.

FIGURE 10. This is the graphic representation of Figure 9 showing the change in in vivo resting LES pressure during the experimental period for each of the 6 groups. There were no 3 week recovery studies done on the prostaglandin E₂ and F₂α groups.

FIGURES 11 - 16. For each ring specimen studied, i.e. 2 distal, LES, 2 proximal, 4 proximal, 6 proximal, and 8 proximal, the total, basal, active, passive, and basal-active forces generated at each stretch length (12, 24, 28, 30, 32, and 34 mm) are shown. The LES ring corresponds to that ring containing the suture placed under fluoroscopy. These Figures represent the non-acid control group.

FIGURES 17 - 21. Here, the same data in Figures 11 through 16 is presented differently. The ring specimens (HP or "high pressure" representing the LES, 8P representing the 8th ring proximal to the LES, etc.) are compared with one another for a given force production. In order, comparisons in the development of basal, total, passive, active, and basal-active forces are presented.

FIGURES 22. The mean maximal active force for each of the esophageal rings (8 proximal through 2 distal) is shown.

FIGURE 23. Here is displayed the length at which the maximal active force is developed by each esophageal ring specimen (8 proximal through 2 distal).

FIGURES 24 - 29. These figures form the companion studies (after induction of esophagitis) to Figures 11 through 16. For each ring specimen studied, i.e. 2 distal through 8 proximal, the total, basal, active, passive, and basal-active forces generated at each stretch length (12, 24, 28, 30, 32, and 34 mm) are shown.

FIGURES 30 - 33. Here are shown the basal, passive, basal-active, and active forces generated by each of the rings tested (8 proximal through 2 distal) both before and after induction of esophagitis, i.e. on days 1 and 5 respectively. The graphs labeled "control" are identical to Figures 17, 19, 21, and 20 in order of appearance. The graphs labeled "esophagitis" are derived from the same data used to arrange Figures 24 through 29.

FIGURE 34. The maximal active force of each individual animal developed at the level of the LES (HP) and at the fourth proximal ring is shown by either a dot or square. Dots or squares connected by a line indicate that both rings come from the same animal. The 4 horizontal lines drawn in the midst of each sample represent that sample's mean maximum active force. "Control" indicates the non-acid control group while "esophagitis" indicates the acid control group on day 5.

FIGURES 35 - 39. The purpose of these graphs is to correlate the degree of histologic damage secondary to HCl infusion with maximum active force (MAF) production. In each graph, the control data (non-acid) is given for comparison with the acid control group on day 5 (labeled esophagitis) and for comparison with those cats of the acid control group with MAF greater than 7.85 as well as those with MAF less than 7.85. Passive, basal, total, active, and basal-active forces developed at the LES are displayed. An asterix designates those points on the force curves that differ significantly ($p < .05$) from the control points of identical stretch length.

FIGURES 40 - 44. These are analagous to Figures 35 through 39 except that passive, basal, total, active, and basal-active forces developed 4 rings proximal to the LES are displayed. Again, the non-acid control group is compared with day 5 of the acid control group as well as with those of the acid control group with $MAF > 7.85$ and those with $MAF < 7.85$. The asterix shows those points that differ significantly ($p < .05$) from identical stretch lengths of the non-acid control animals.

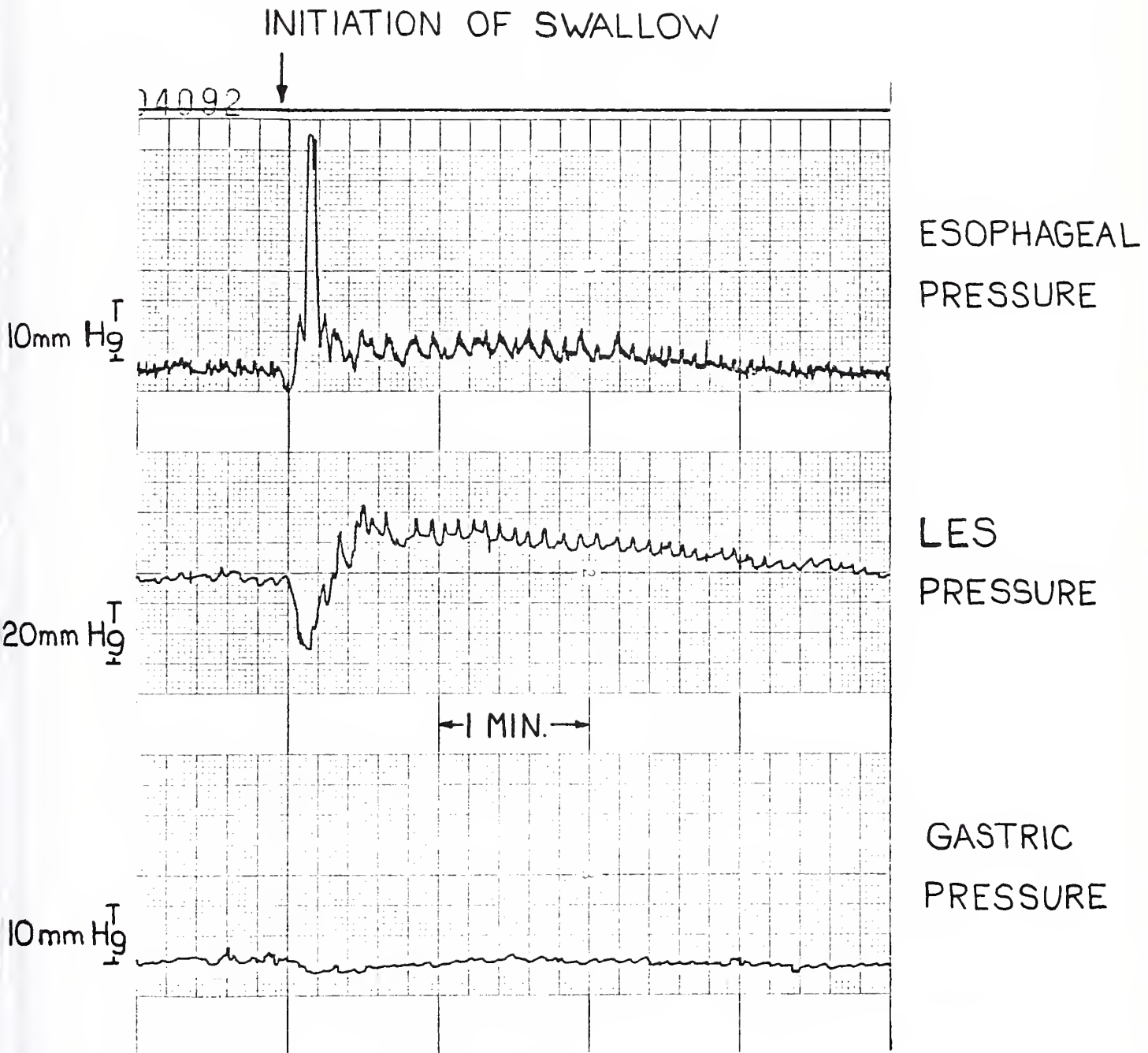
FIGURE 45. This graph displays the total, active, basal, passive, and basal-active forces developed at each stretch length (12, 24, 28, 30, 32, and 34 mm) by the LES of those cats in the high dose indomethacin group.

FIGURE 46. Here, the mean basal, active, and total forces generated at a stretch length of 32 mm by the non-acid control group, the acid control group and the indomethacin (4 mg/kg) group are displayed for comparison. There was no significant ($p < .05$) difference between the acid control group and the indomethacin group for any of these in vitro forces.

FIGURE 47. This graph displays the total, active, basal, passive, and basal-active forces developed at each stretch length by the LES of those cats in the prostaglandin E2 group.

FIGURE 48. This graph displays the total, active, basal, passive, and basal-active forces developed at each stretch length by the LES of those cats in the prostaglandin F2 α group.

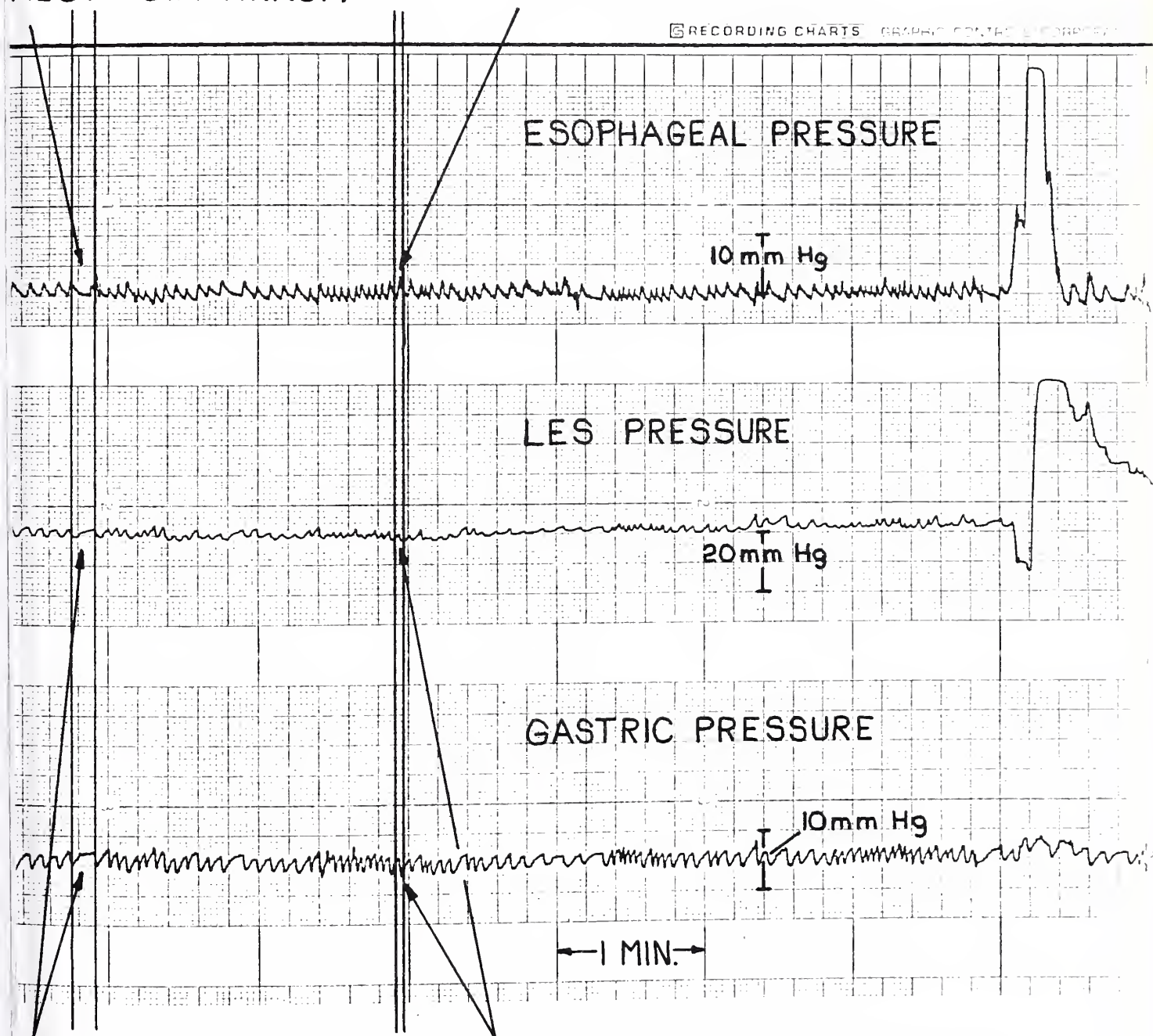
FIGURE 49. Here is shown the mean percentage of ^3H -thymidine labeled cells (from the bottom two layers of squamous mucosa) from suction biopsies at 2 cm and 4 cm proximal to the LES on days 1, 5, and 25.



CURVE SLOPES DOWN
(DECREASE PRESSURE)
WITH INSPIRATION
ABOVE DIAPHRAGM

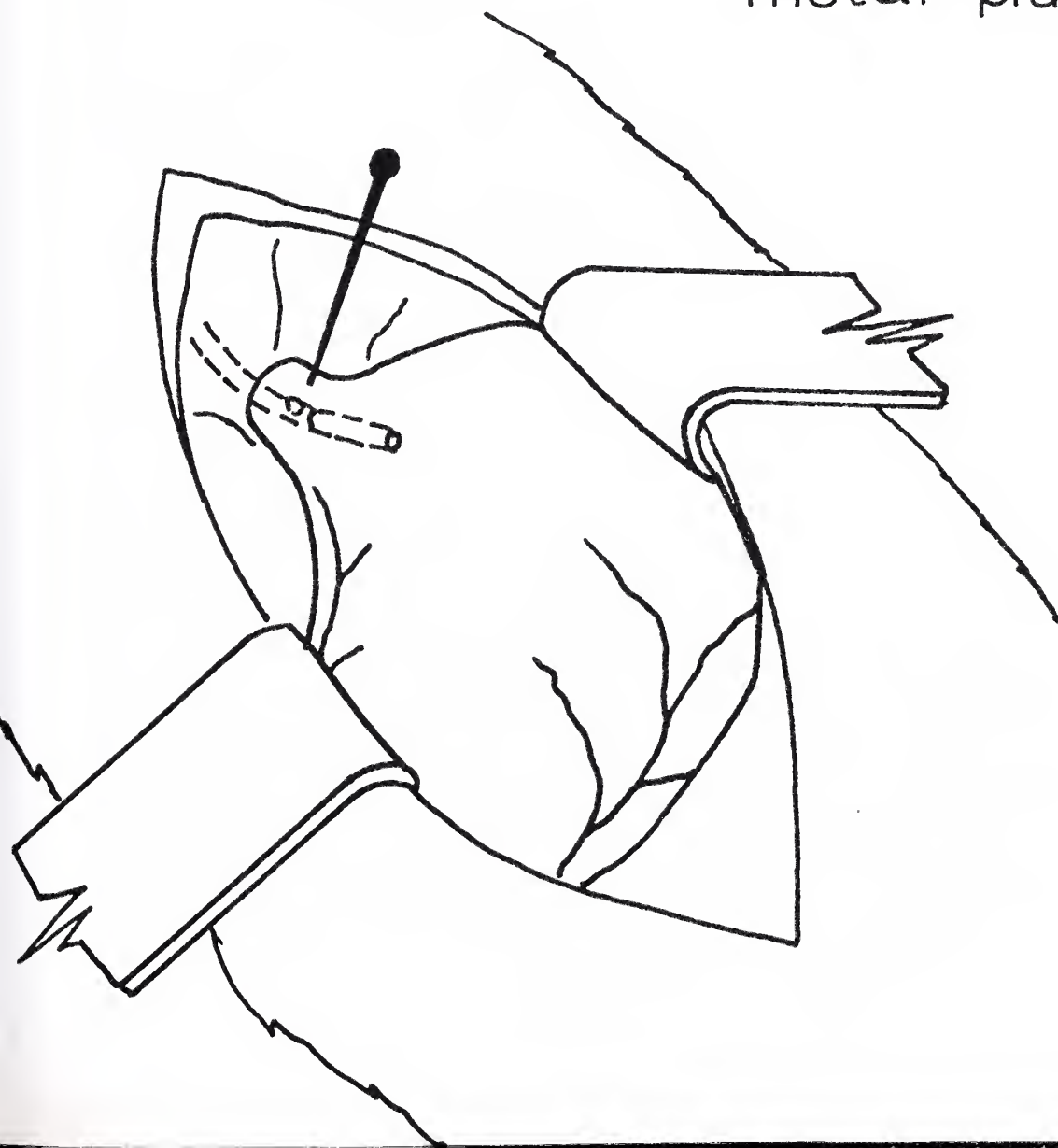
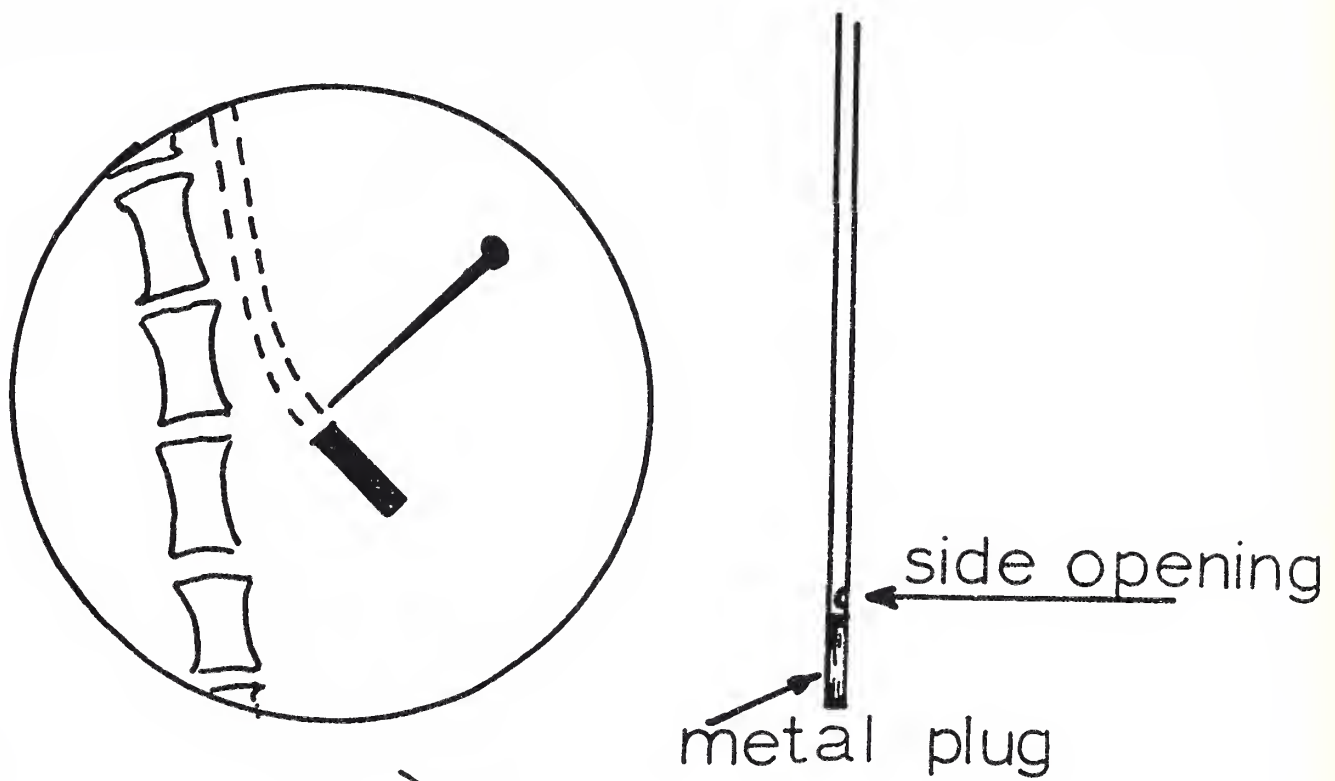
CURVE SLOPES UP (INCREASE
PRESSURE) WITH EXPIRATION
ABOVE DIAPHRAGM

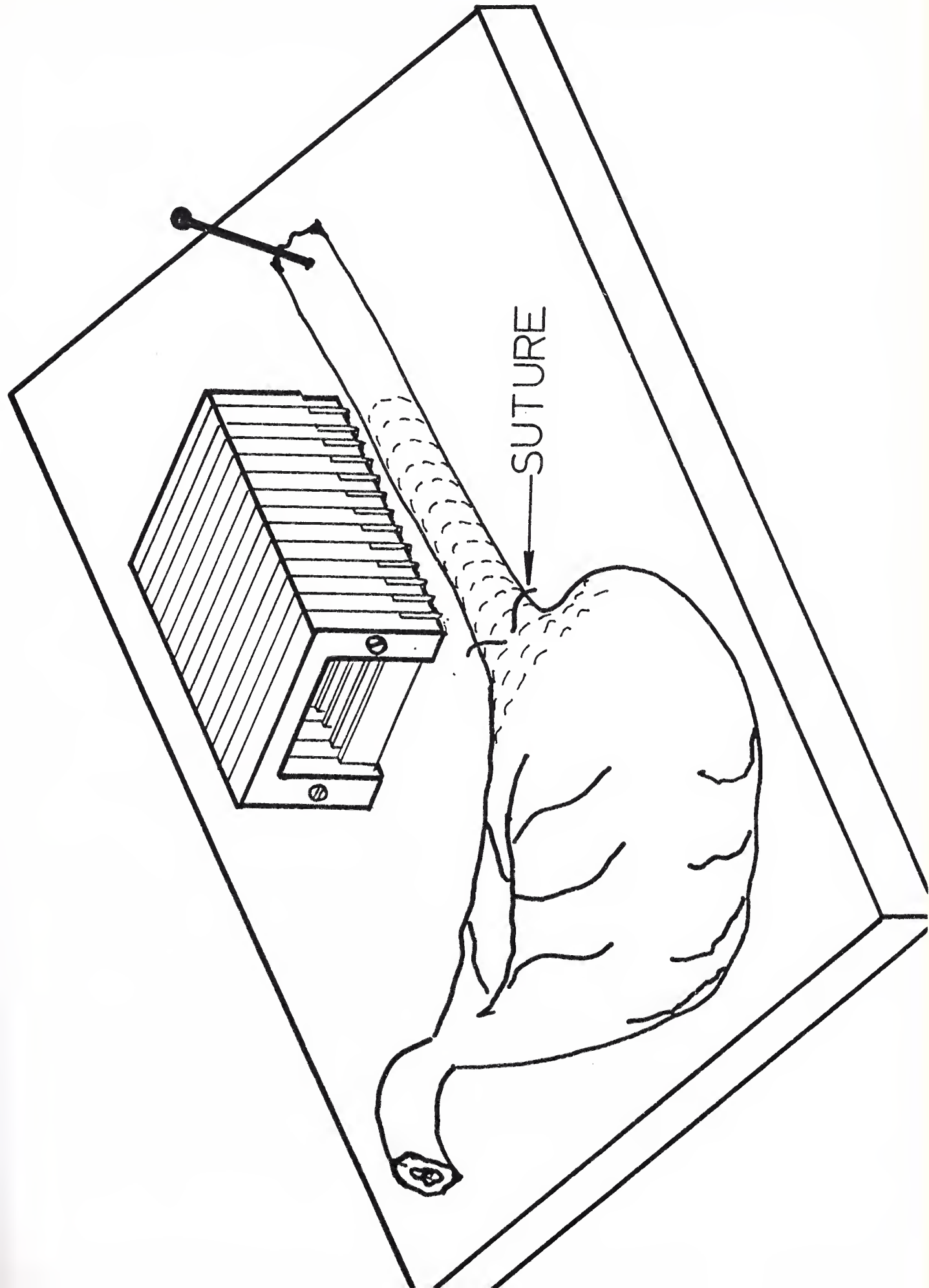
RECORDING CHARTS GRAPHIC CENTER CORP.

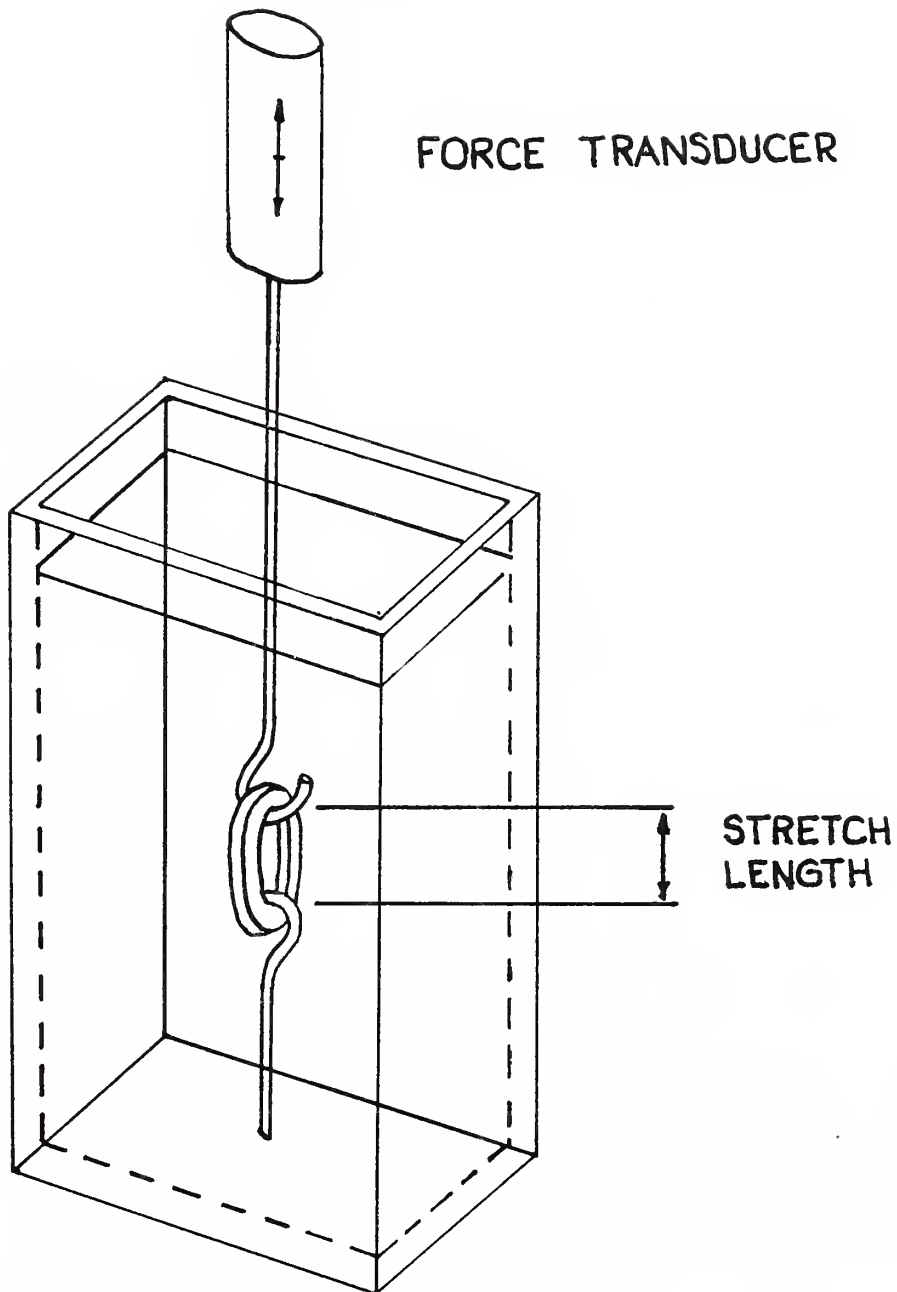


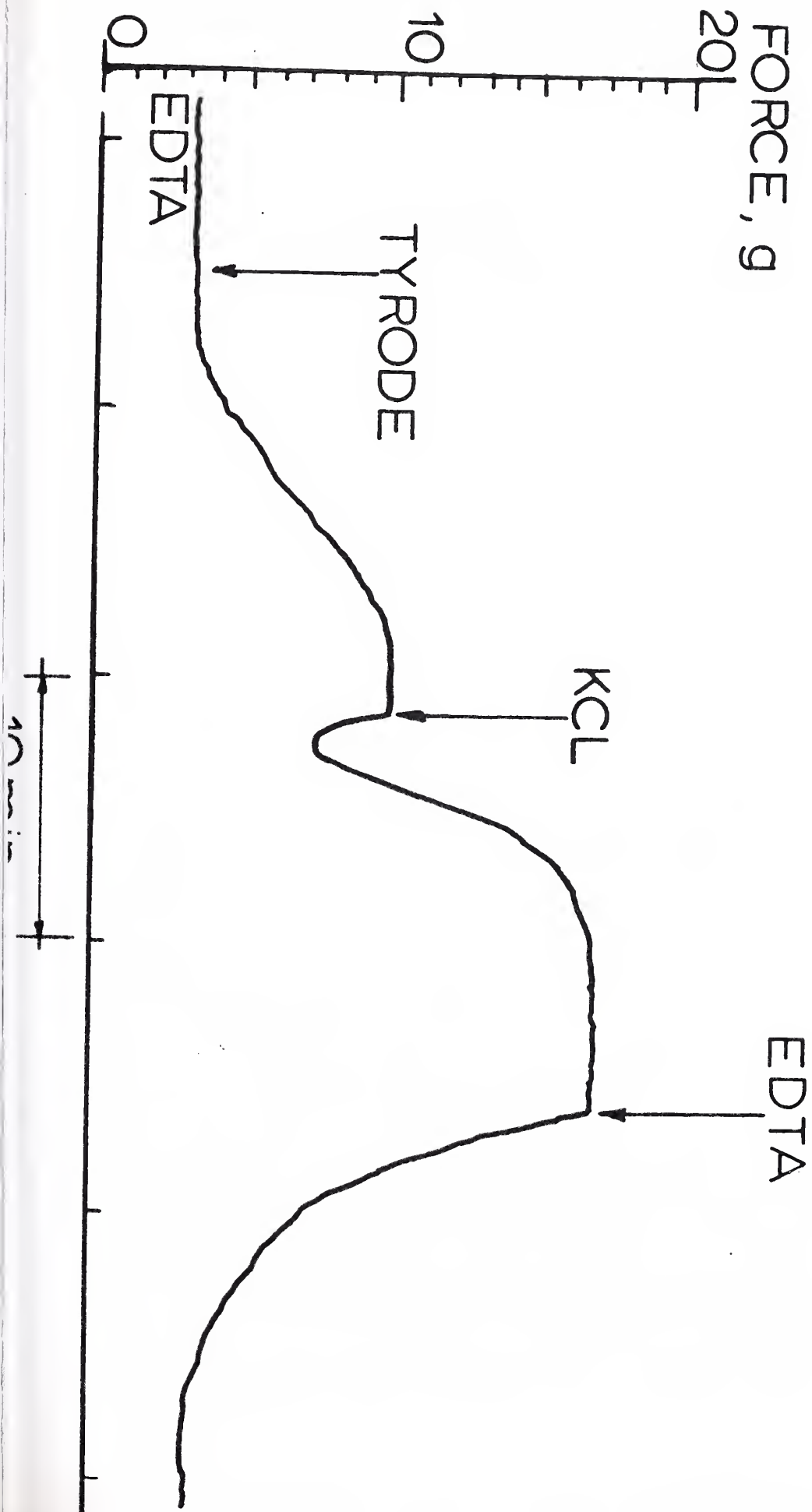
CURVE SLOPES UP
(INCREASE PRESSURE)
WITH INSPIRATION
BELOW DIAPHRAGM

CURVE SLOPES DOWN (DECREASE
PRESSURE) WITH EXPIRATION BELOW
DIAPHRAGM

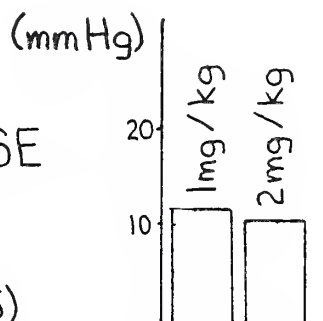




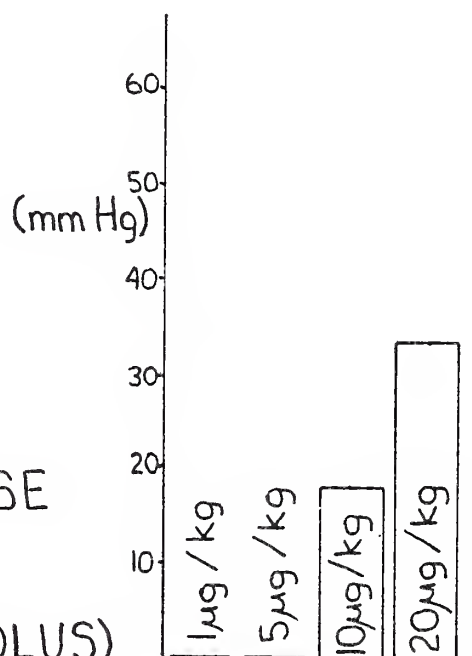




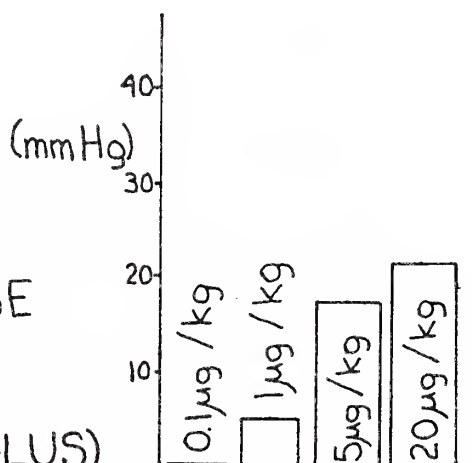
LES PRESSURE DECREASE
WITH
INDOMETHACIN (IV BOLUS)

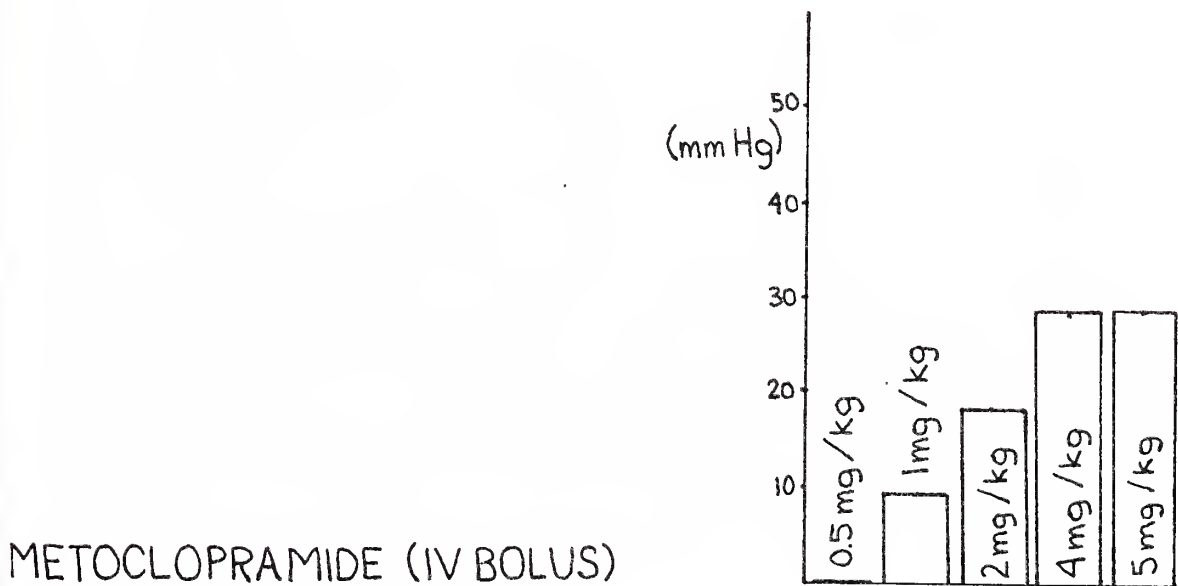
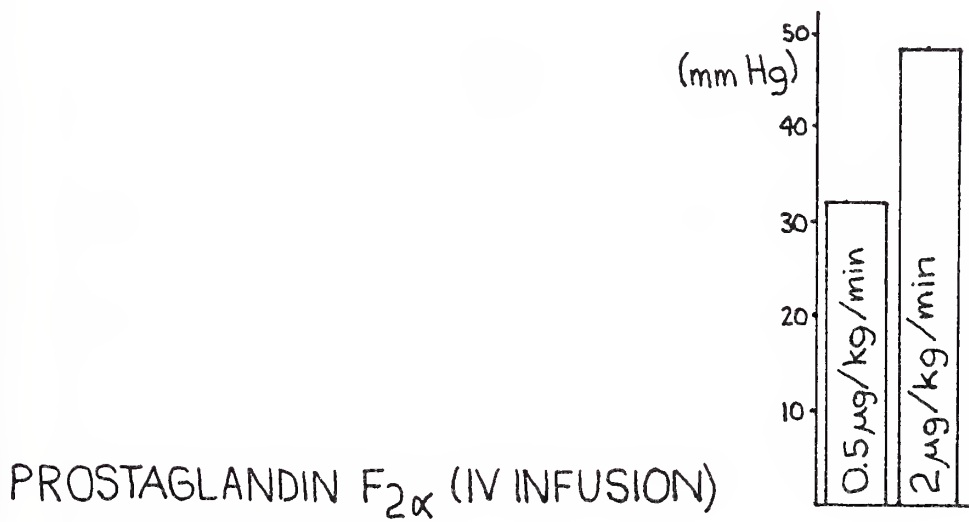
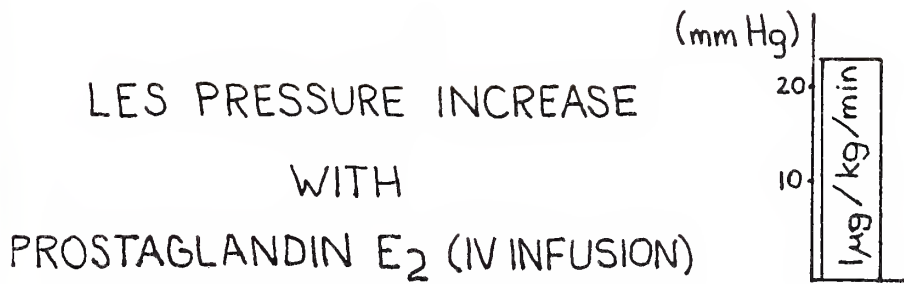


LES PRESSURE INCREASE
WITH
PROSTAGLANDIN $F_{2\alpha}$ (IV BOLUS)



LES PRESSURE INCREASE
WITH
PROSTAGLANDIN E_2 (IV BOLUS)





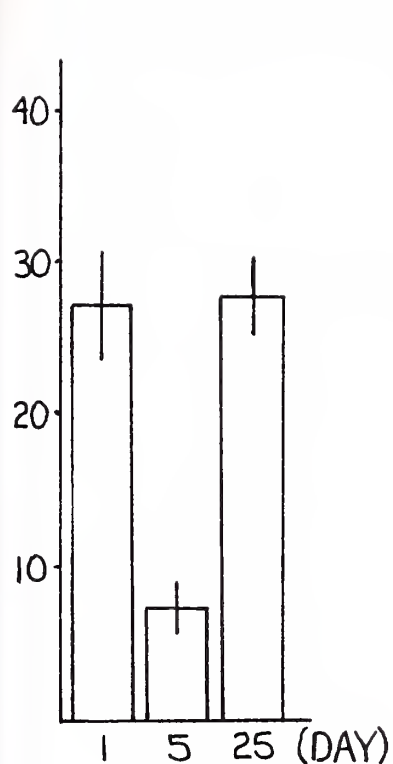
	DAY 1	DAY 5	DAY 25
	PRE-ACID	POST-ACID	POST-RECOVERY
	LES RESTING	LES RESTING	LES RESTING
	PRESSURE	PRESSURE	PRESSURE
	(mmHg \pm S.E.M.)	(mmHg \pm S.E.M.)	(mmHg \pm S.E.M.)
CONTROL	27.5 \pm 3.7 ^b	7.5 \pm 1.7	28.2 \pm 2.6
LOW DOSE INDOCIN	26.6 \pm 1.5 ^a	7.0 \pm 1.3	37.0 \pm 5.6
HIGH DOSE INDOCIN	26.4 \pm 2.8 ^a	10.0 \pm 1.7	31.3 \pm 2.0
METOCLOPRAMIDE	26.5 \pm 6.4 ^c	8.1 \pm 4.1	23.1 \pm 0.9
PROSTAGLANDIN E ₂	23.4 \pm 2.8 ^c	4.7 \pm 1.7	
PROSTAGLANDIN F _{2α}	23.0 \pm 5.8 ^c	4.5 \pm 1.1	

a. $p < .001$ (PRE-ACID VS. POST-ACID)

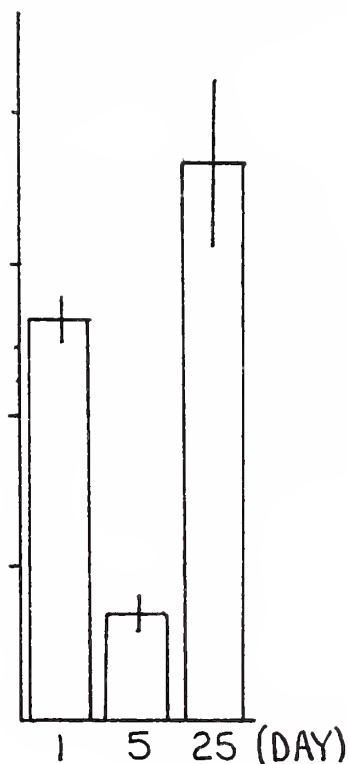
b. $p < .01$

c. $p < .05$

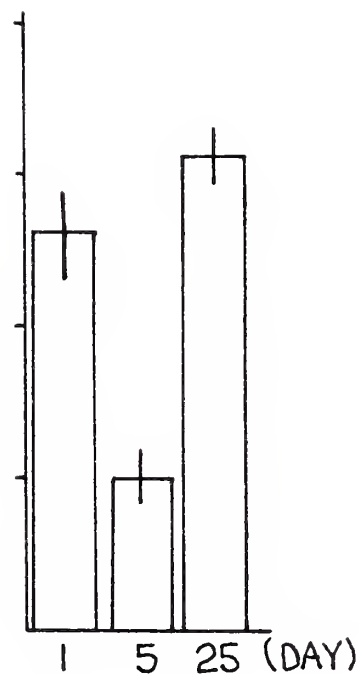
MEAN LES PRESSURE



CONTROL

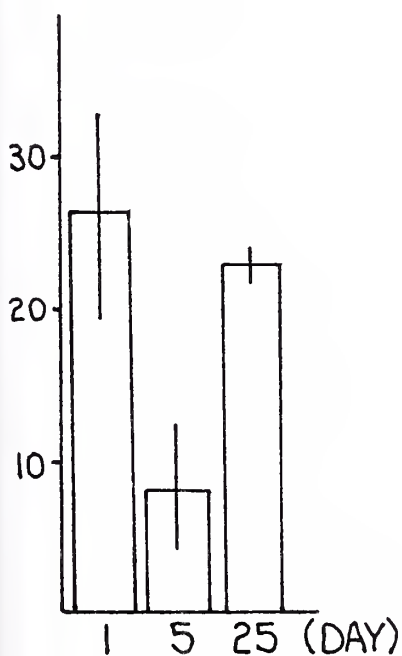


INDOCIN (150 µg/kg)

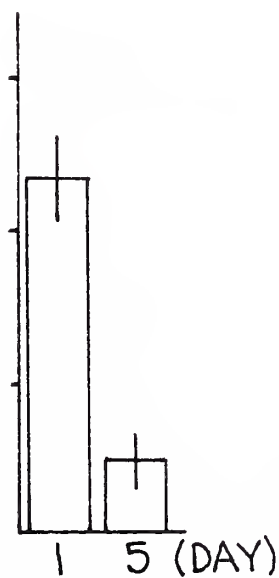


INDOCIN (4 mg/kg)

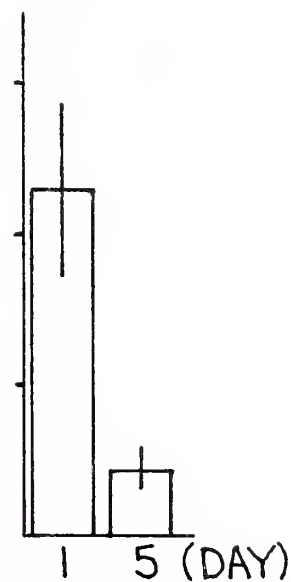
MEAN LES PRESSURE



METOCLOPRAMIDE

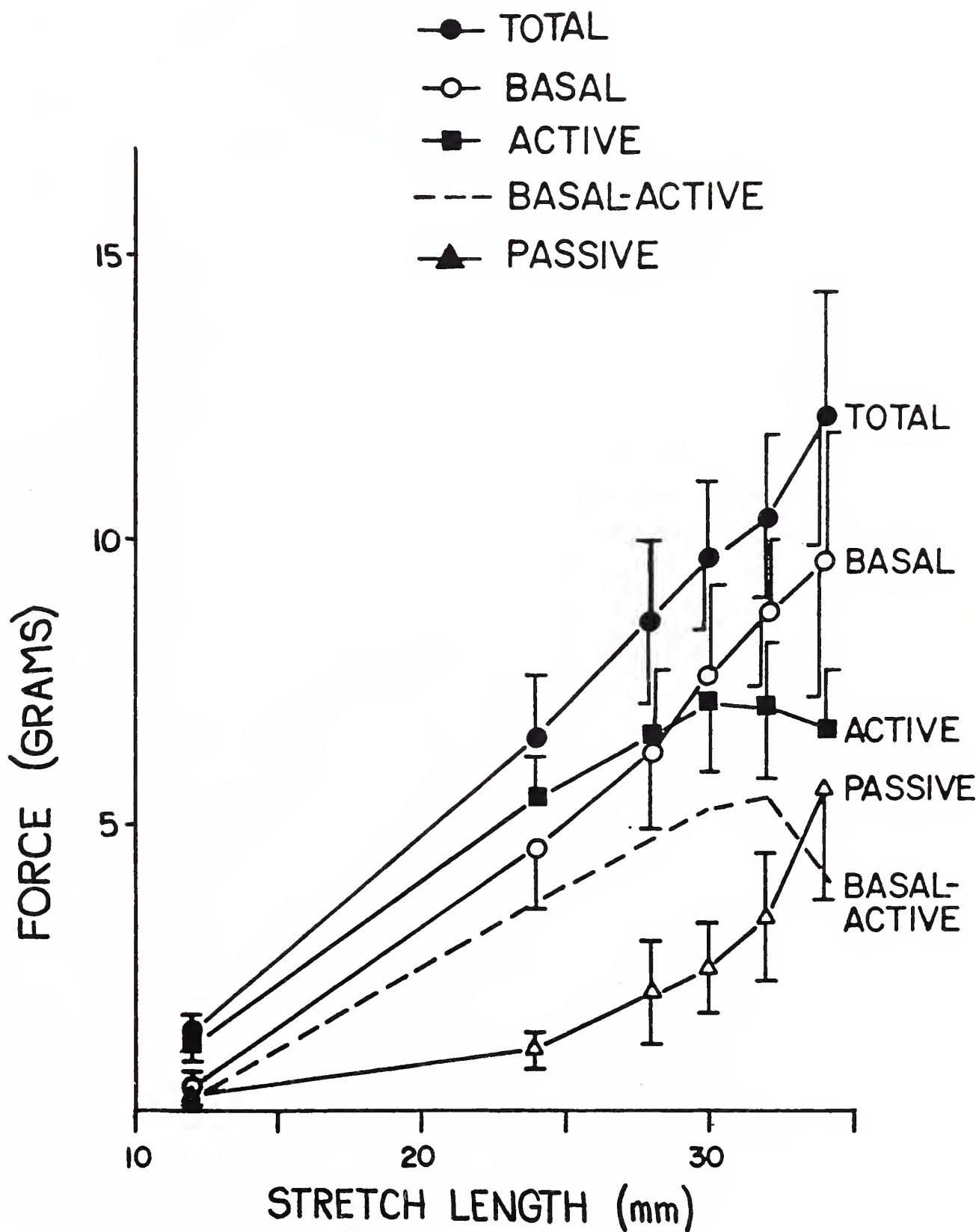


PROSTAGLANDIN E₂

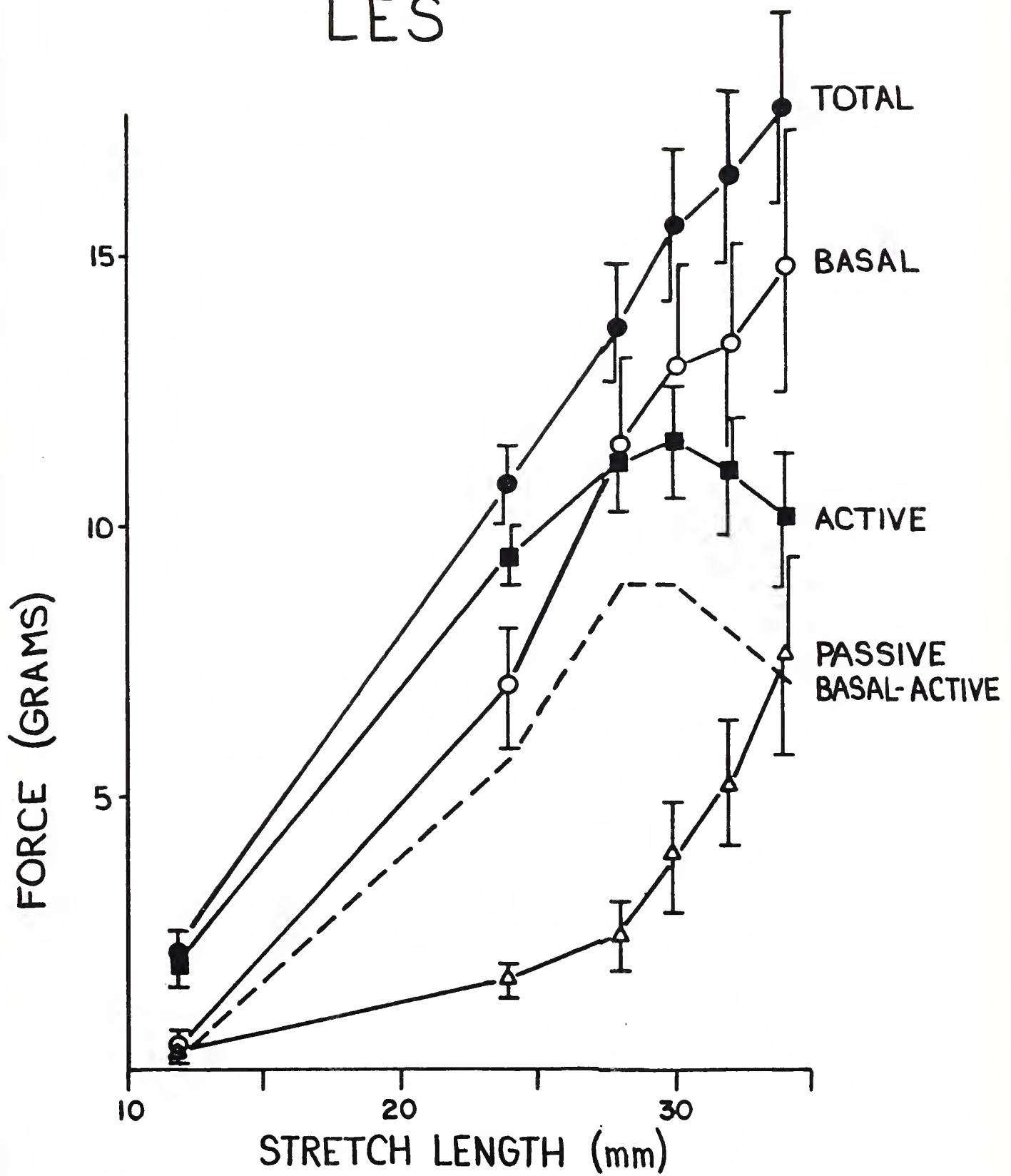


PROSTAGLANDIN F_{2α}

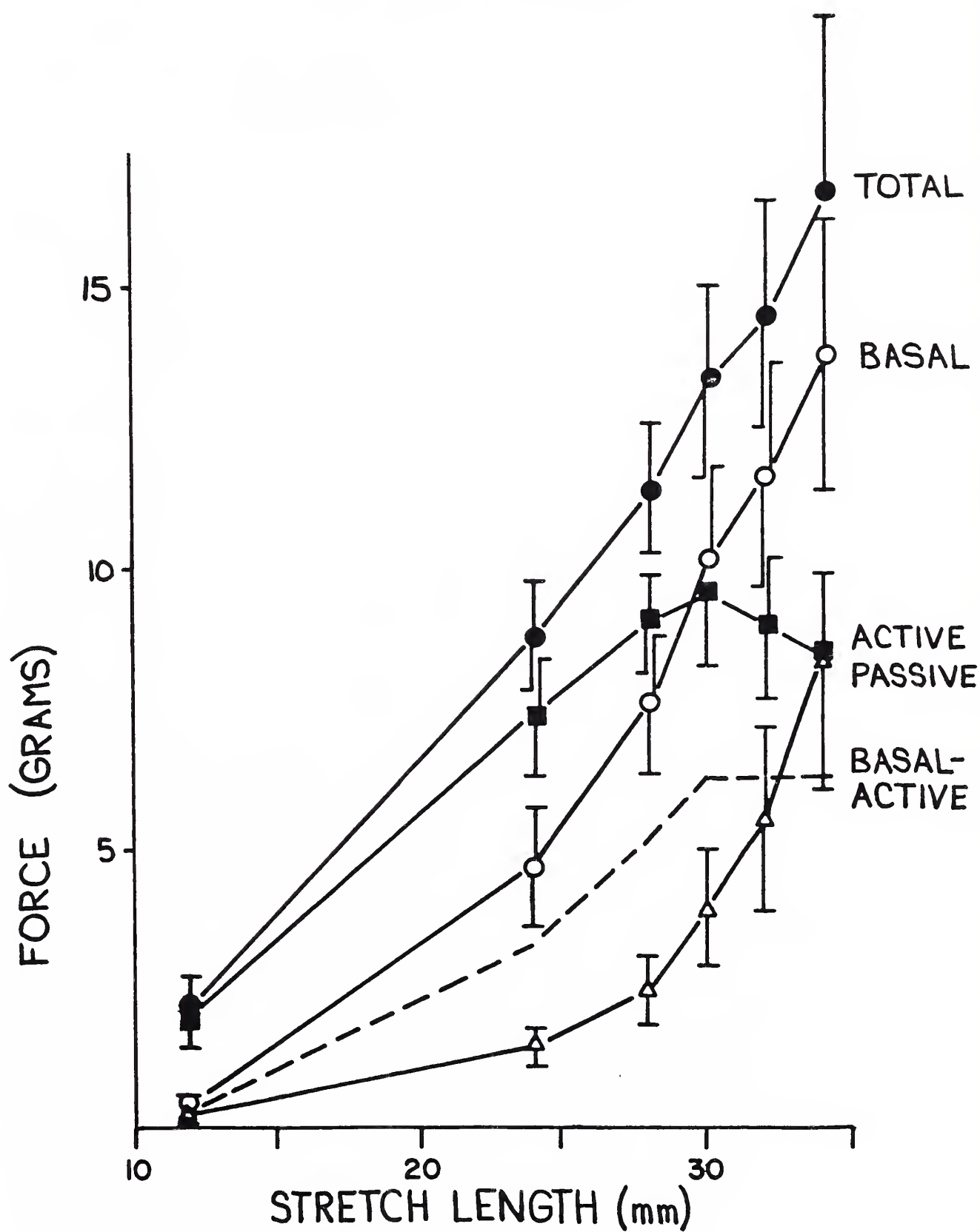
2 DISTAL



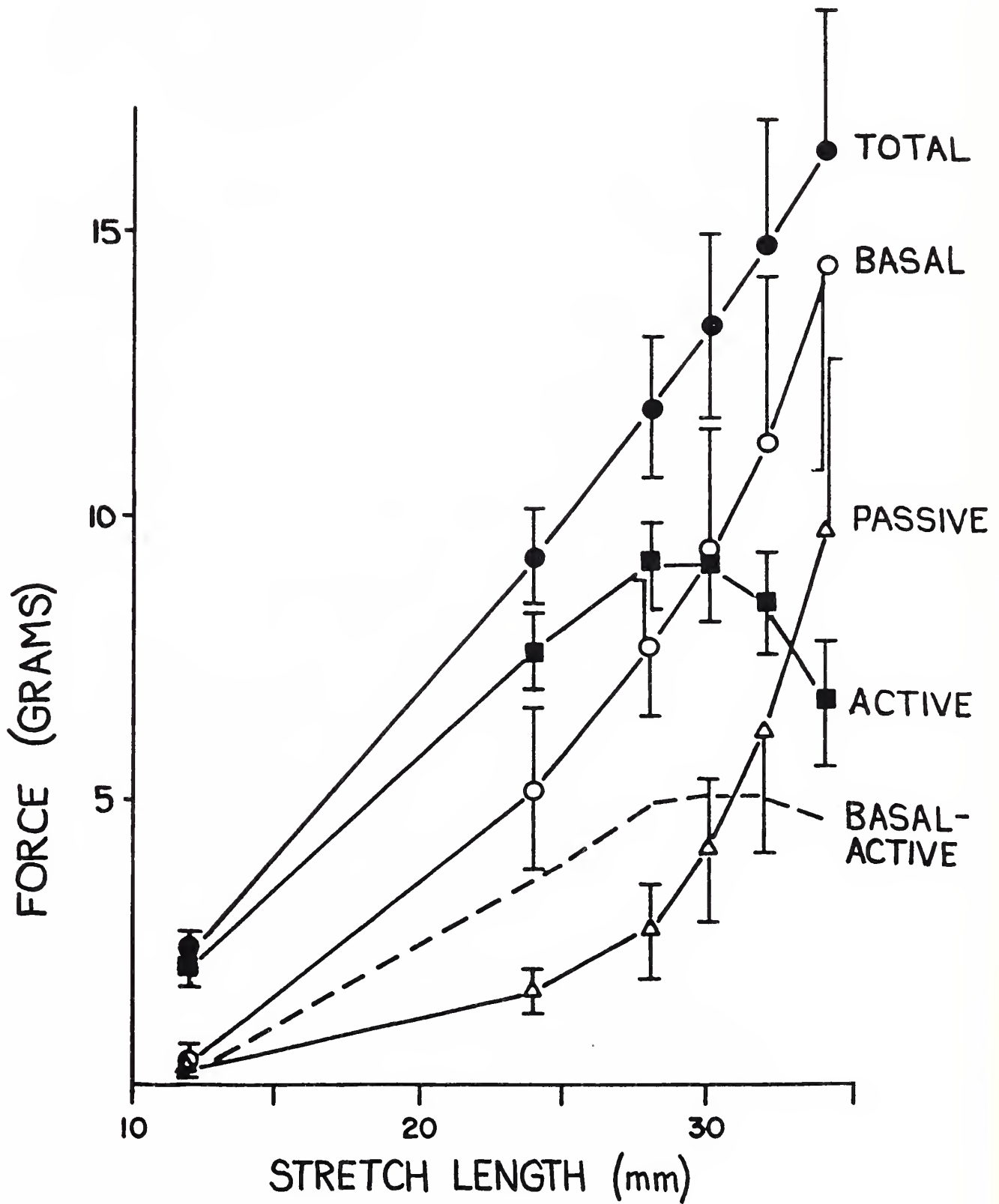
LES



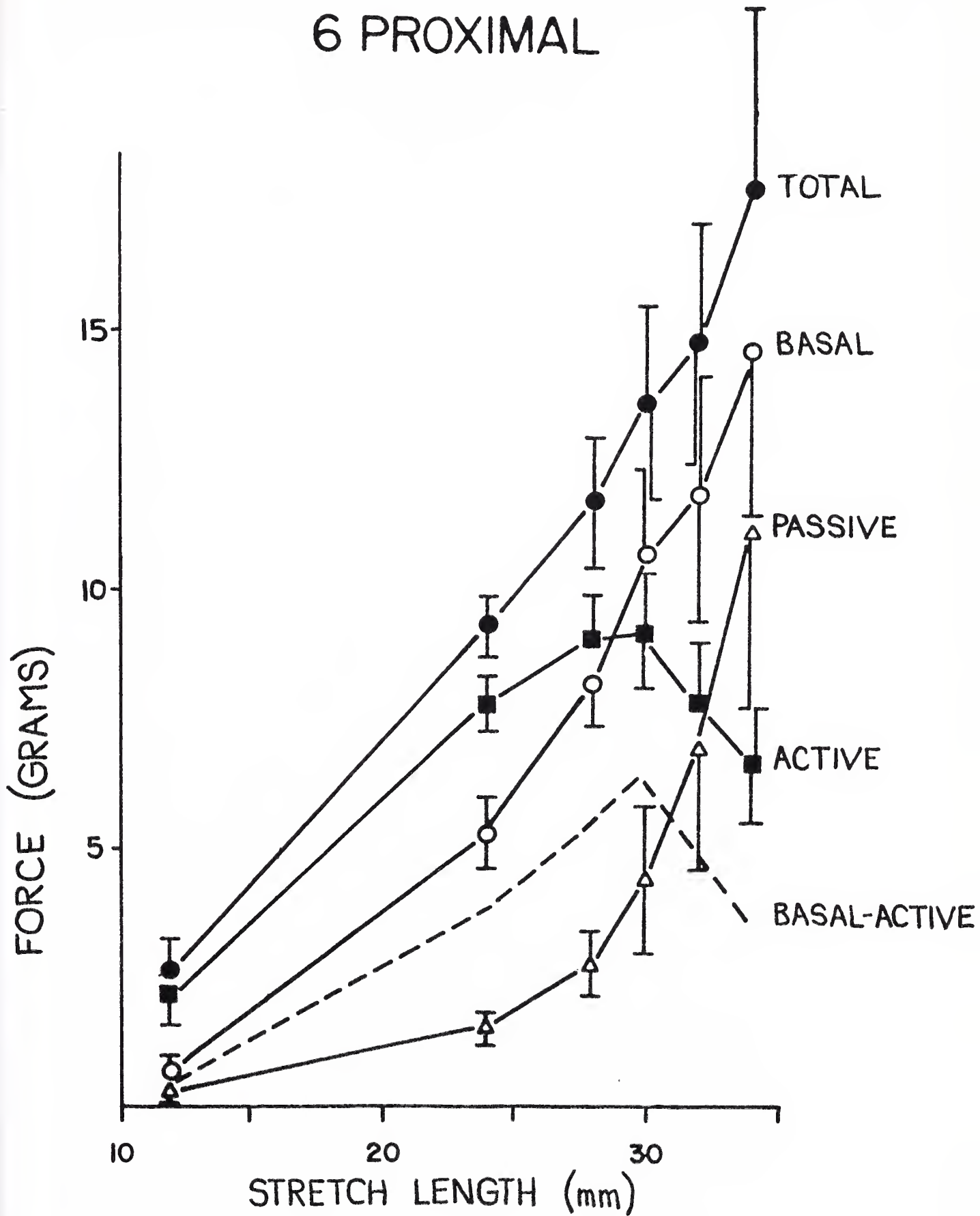
2 PROXIMAL



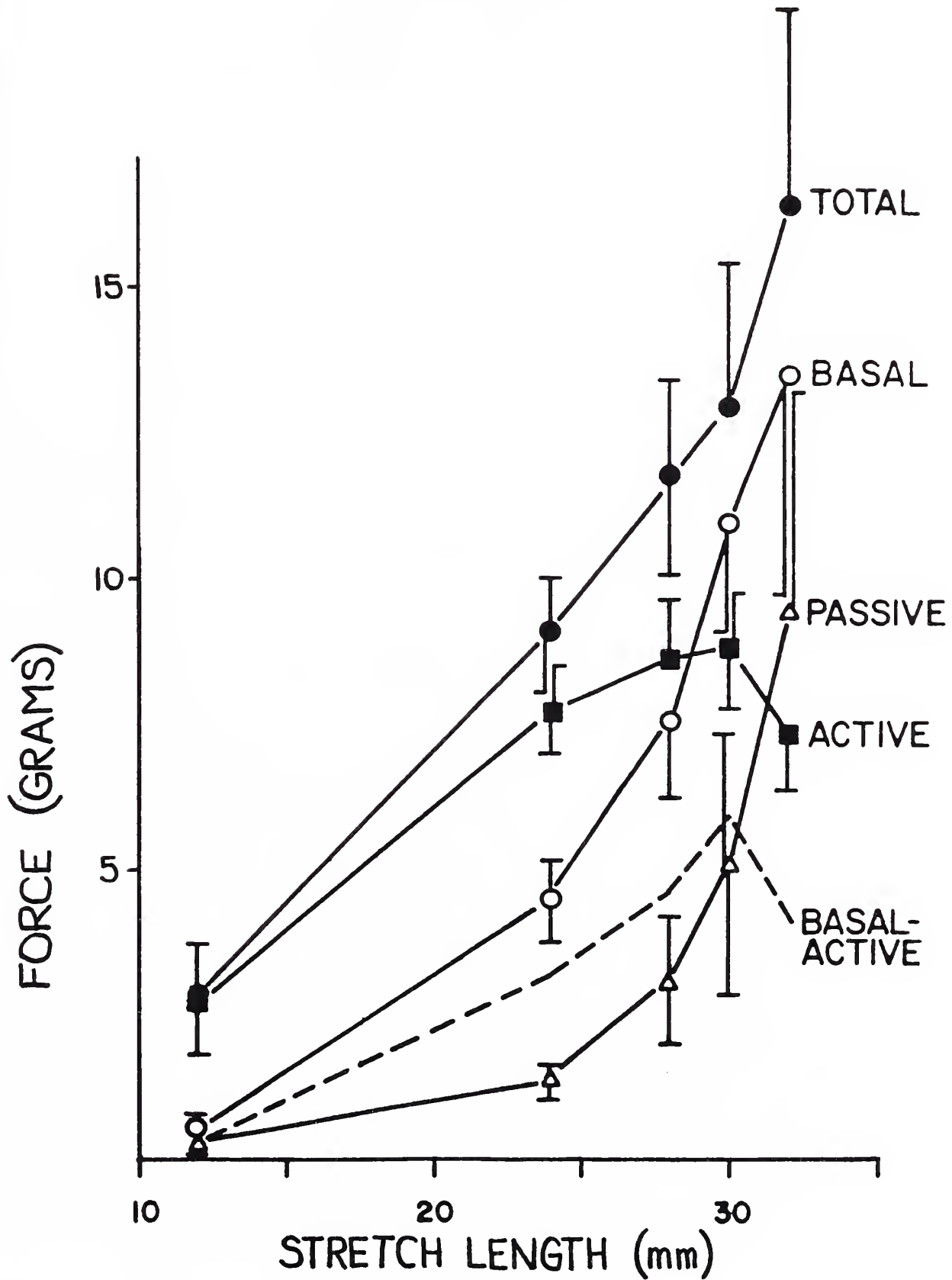
4 PROXIMAL



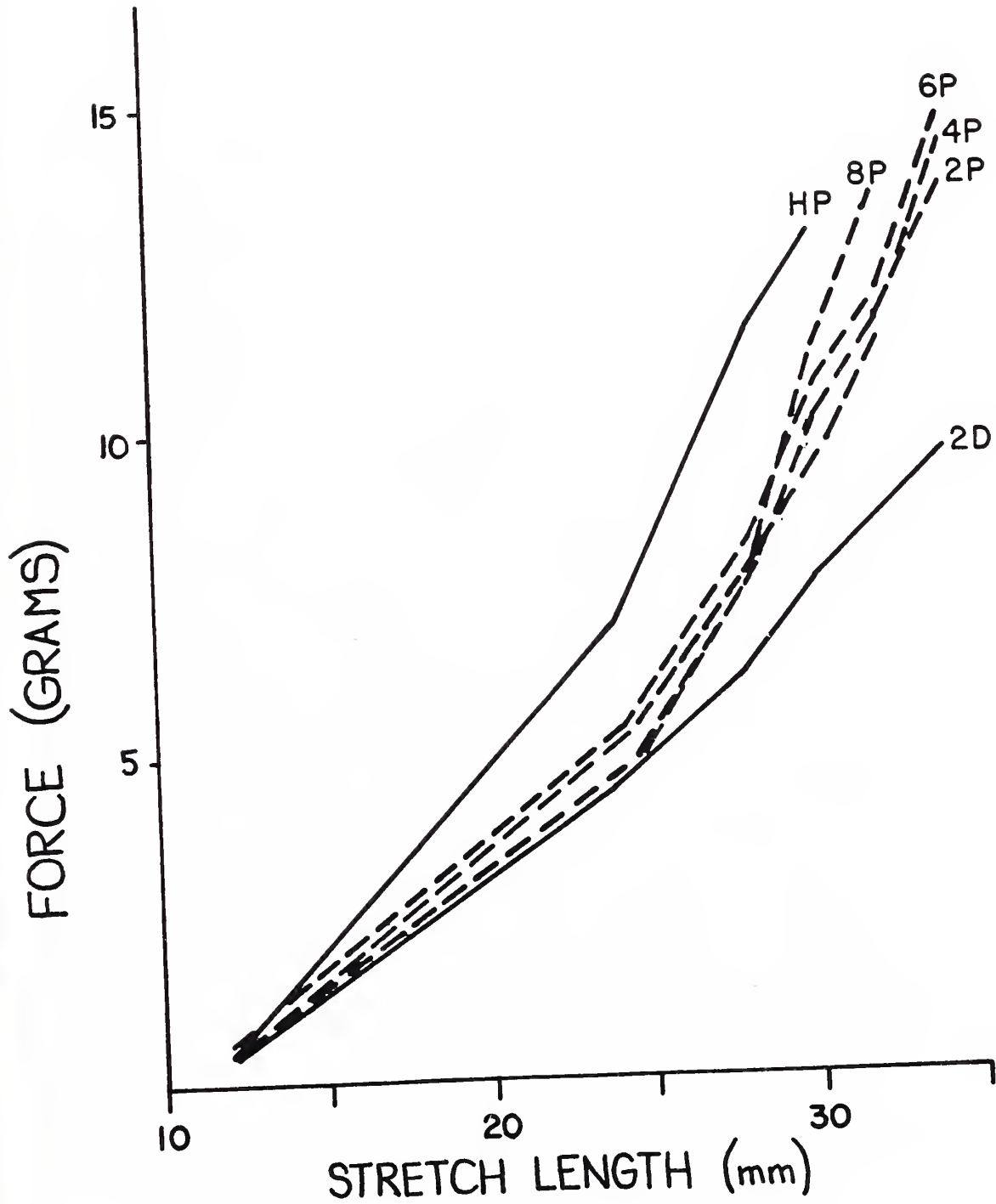
6 PROXIMAL



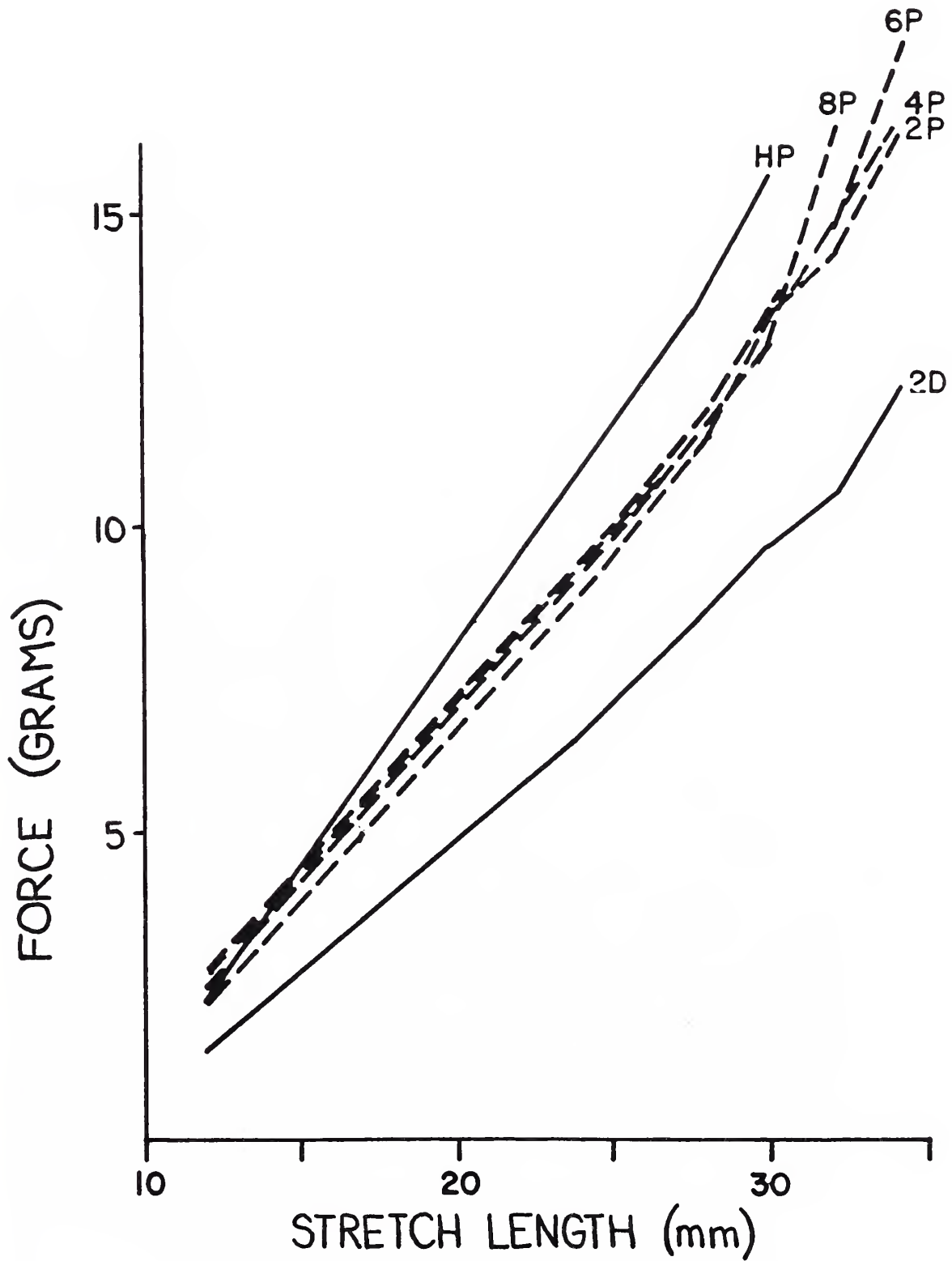
8 PROXIMAL



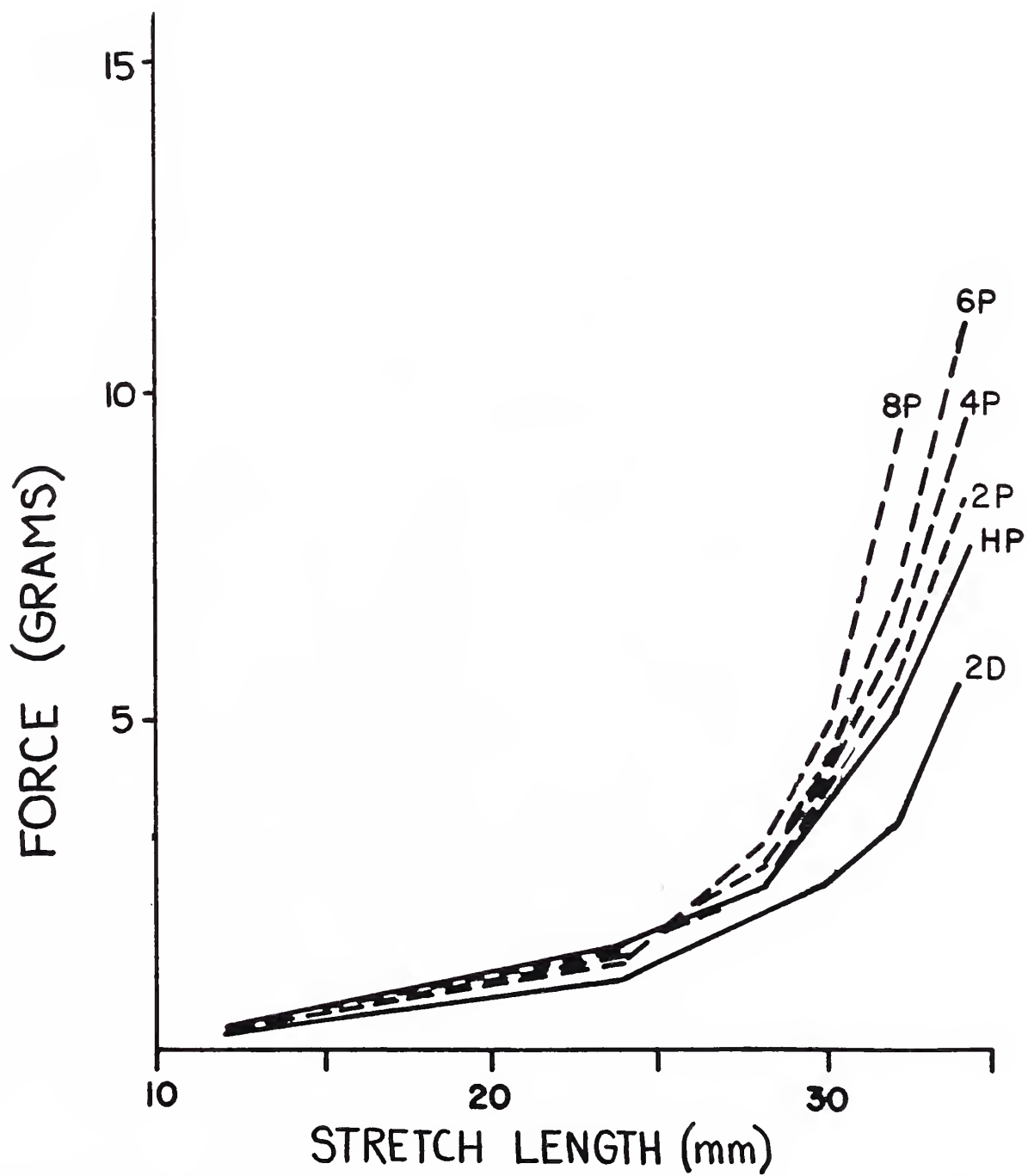
BASAL



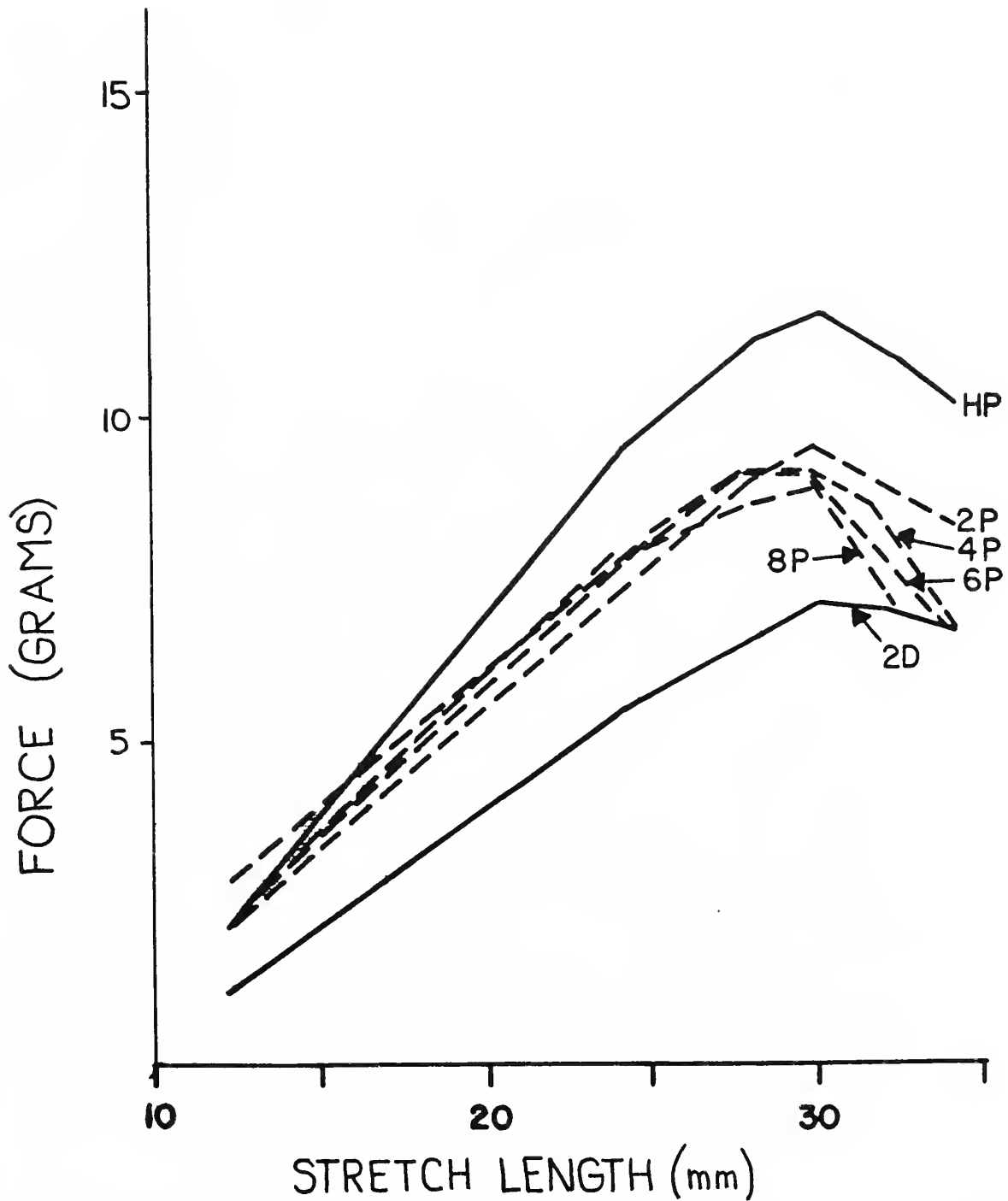
TOTAL



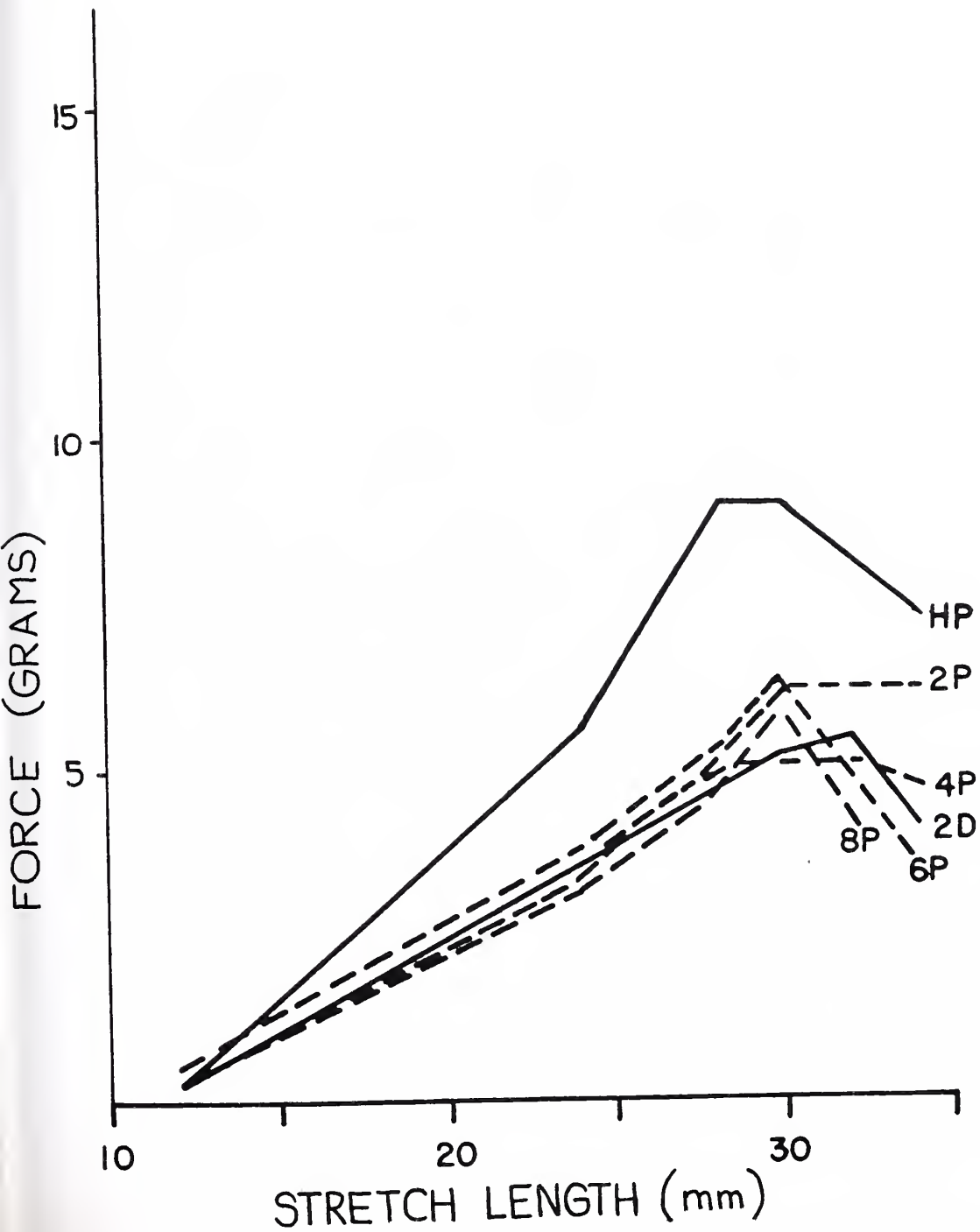
PASSIVE

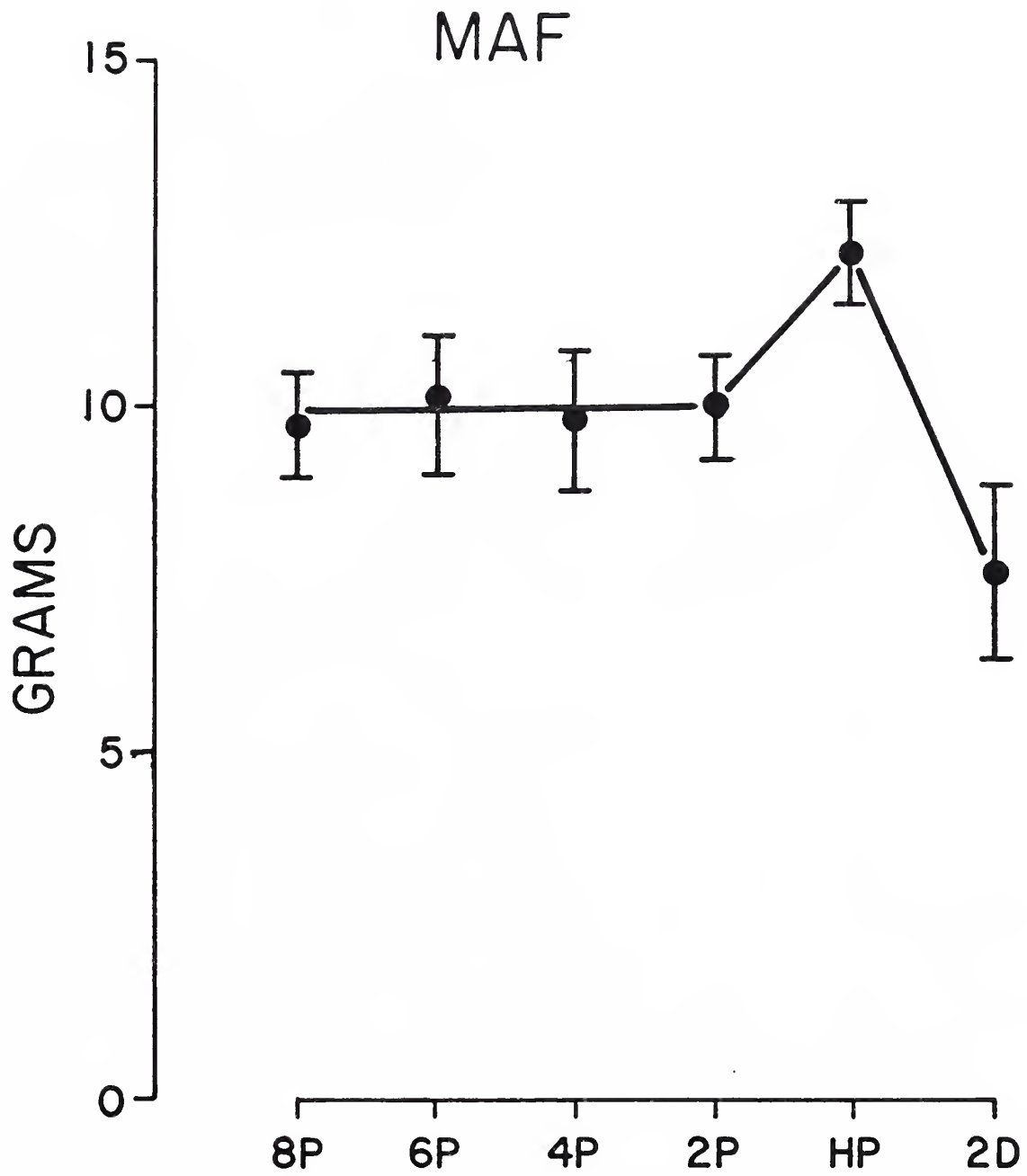


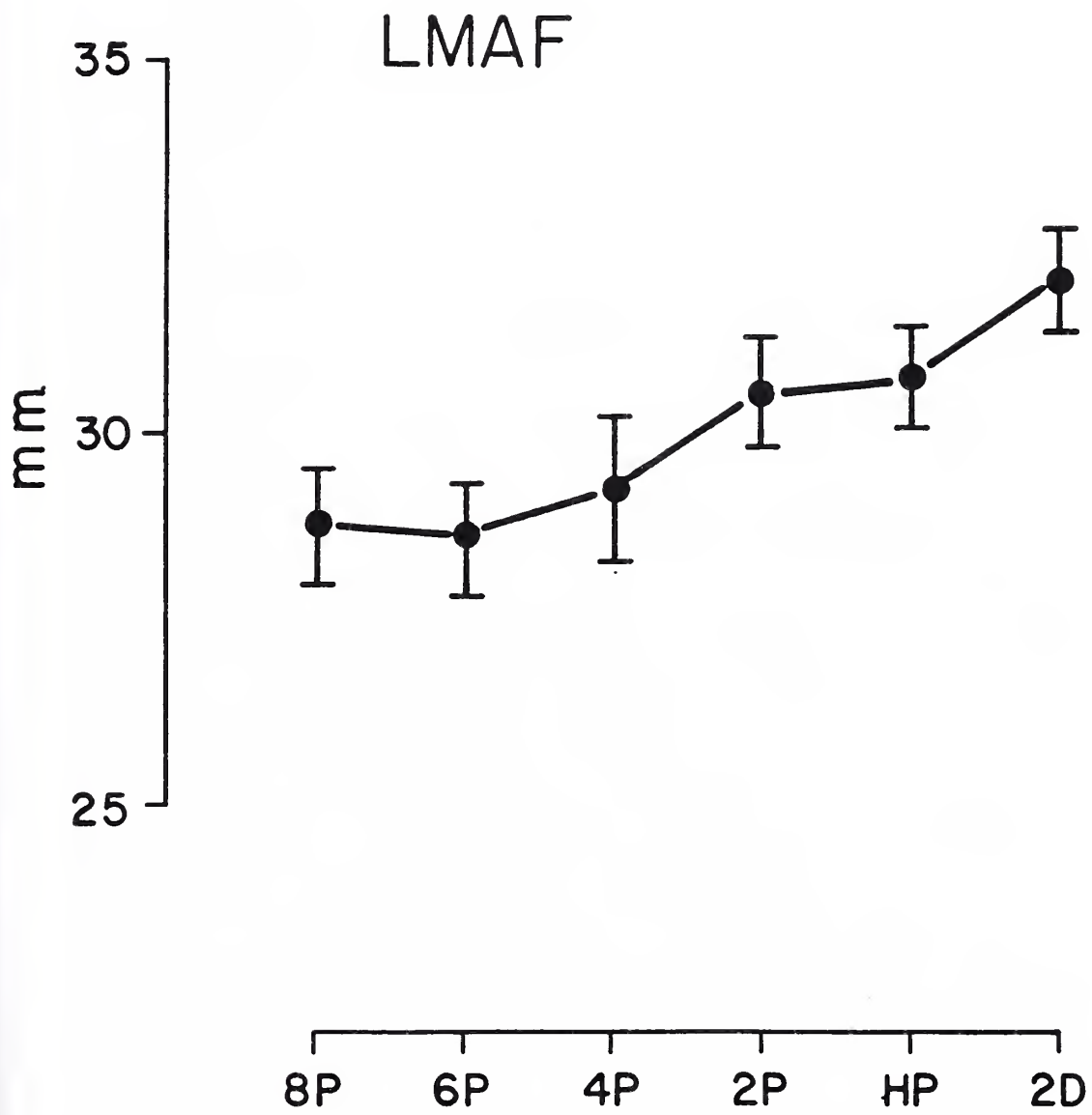
ACTIVE

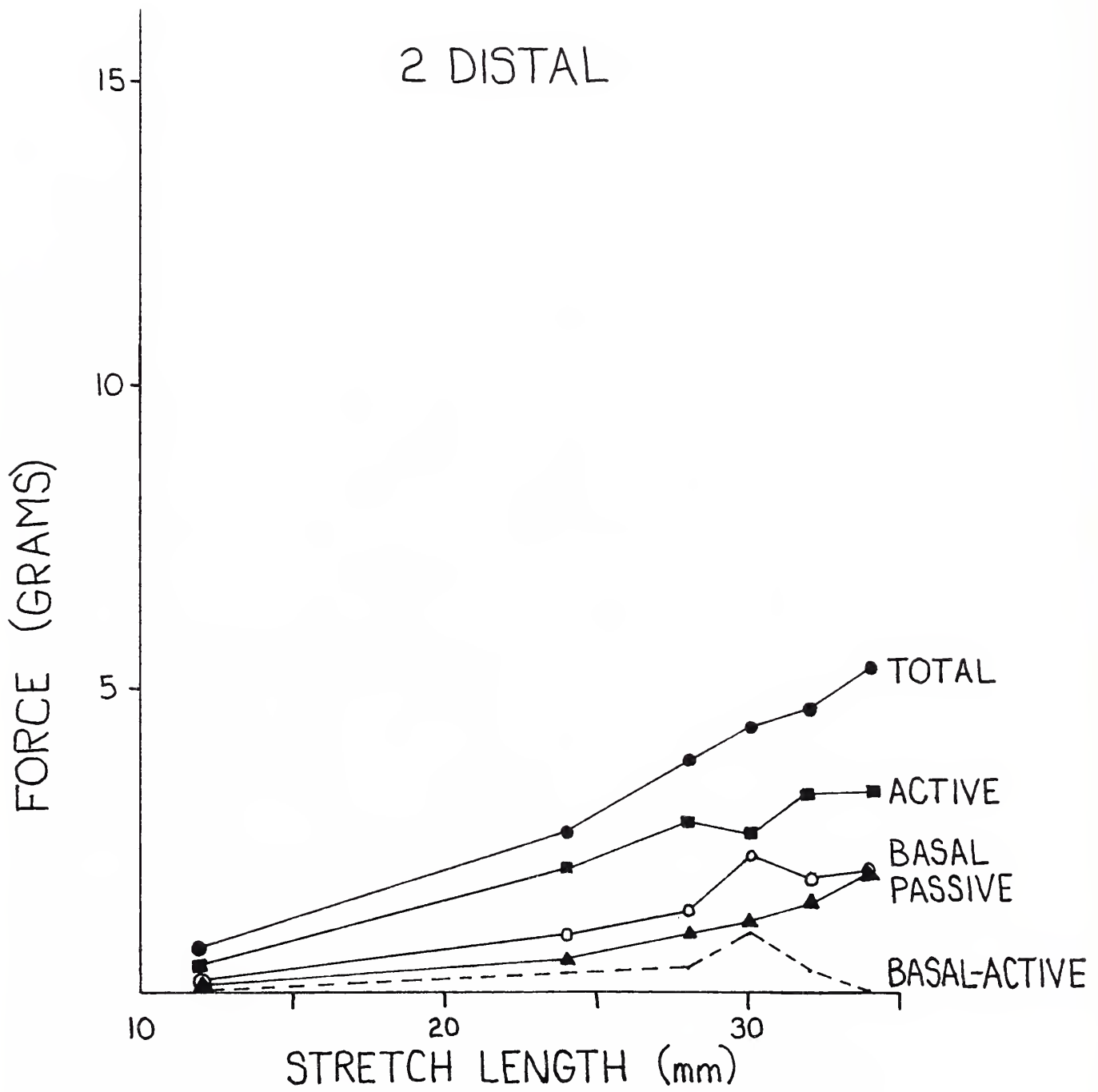


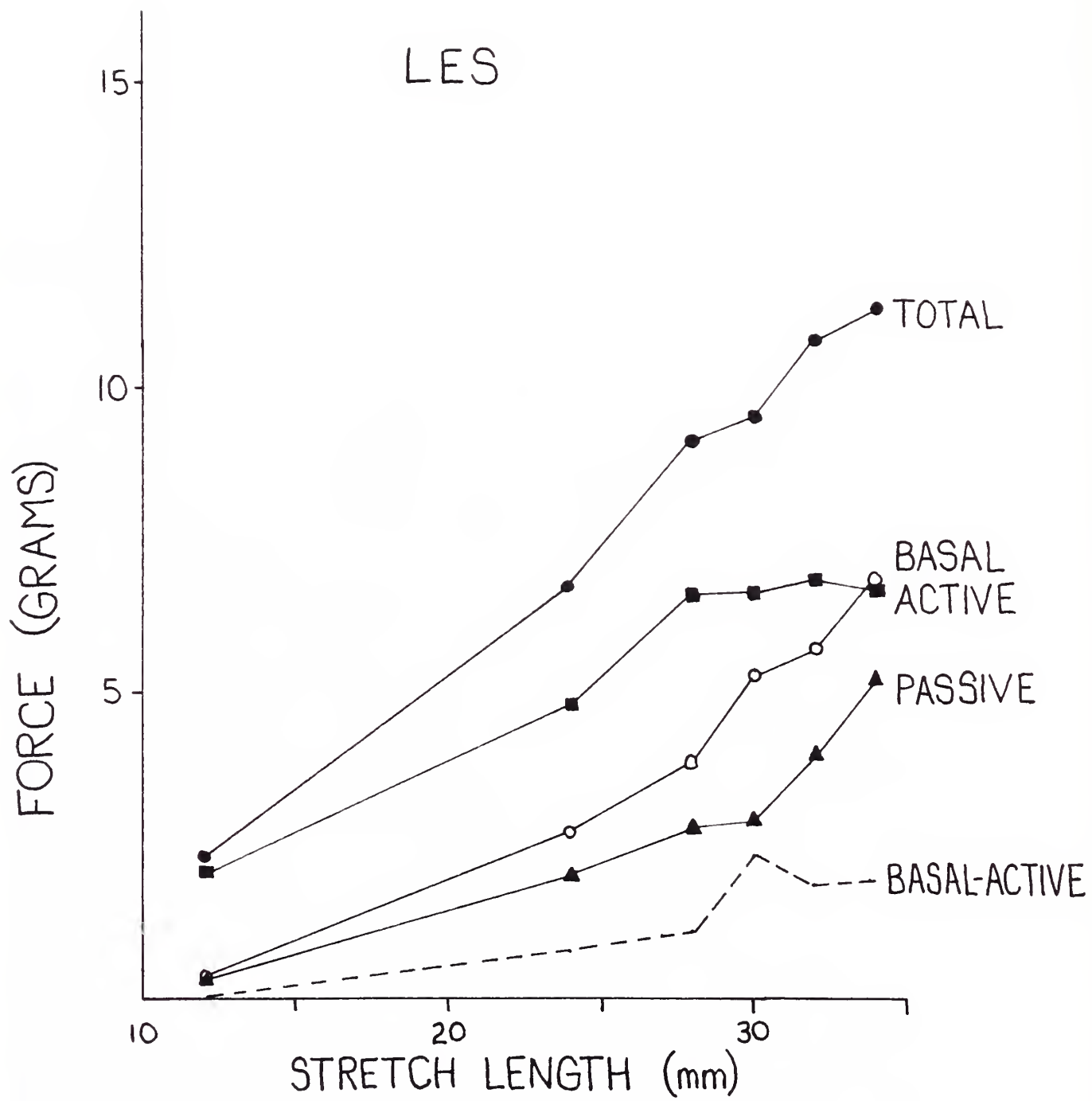
BASAL ACTIVE

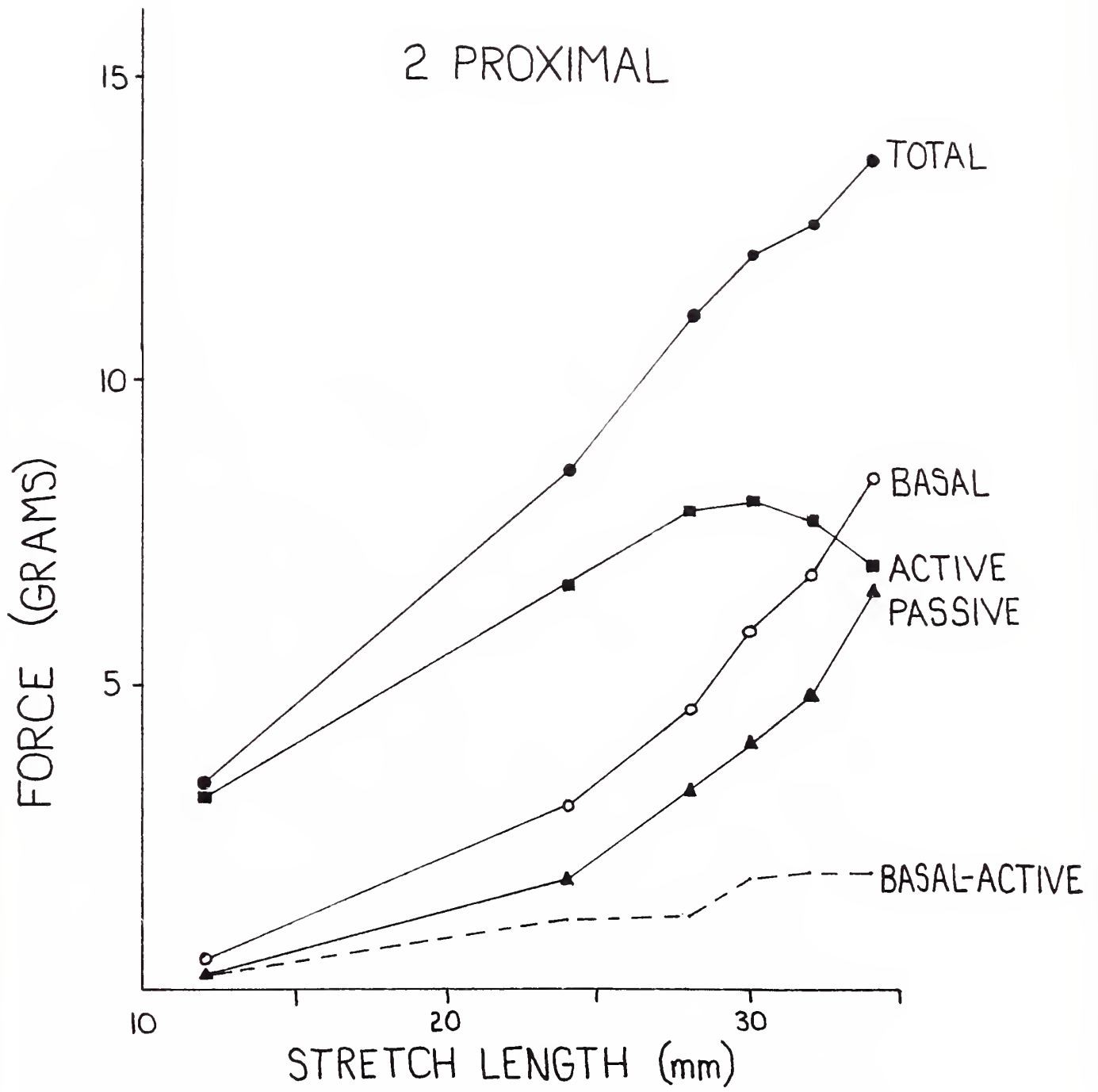


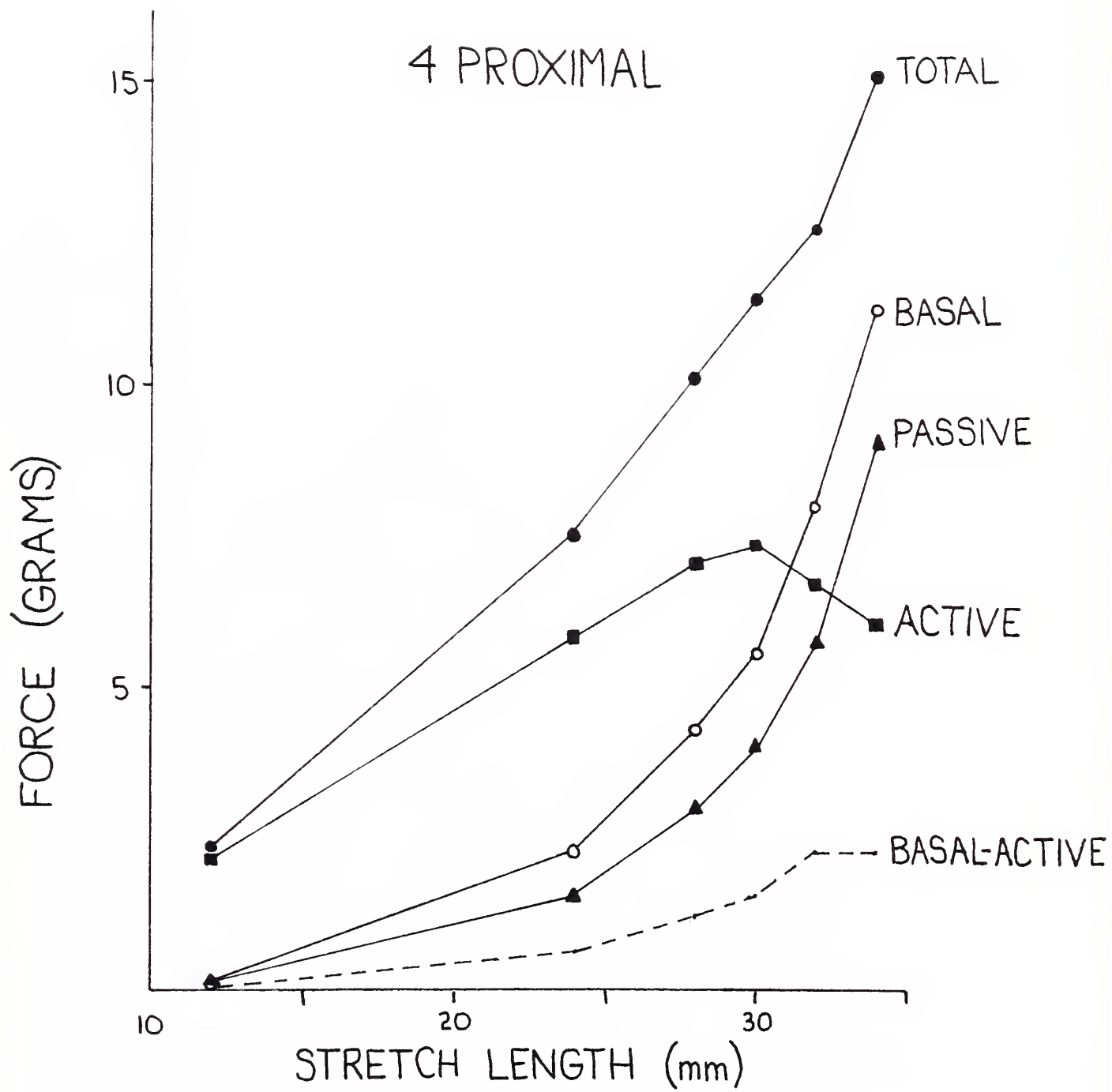


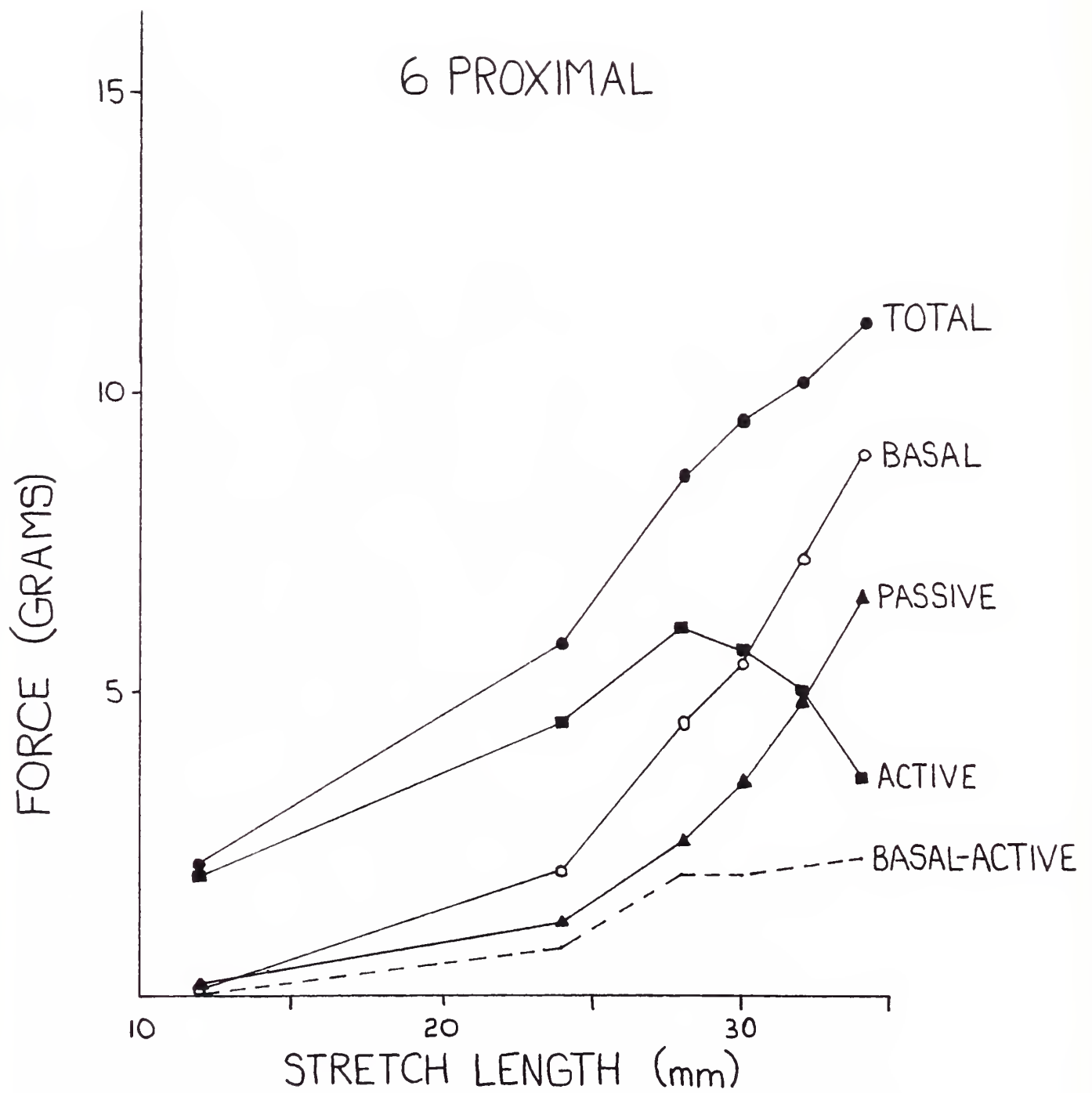


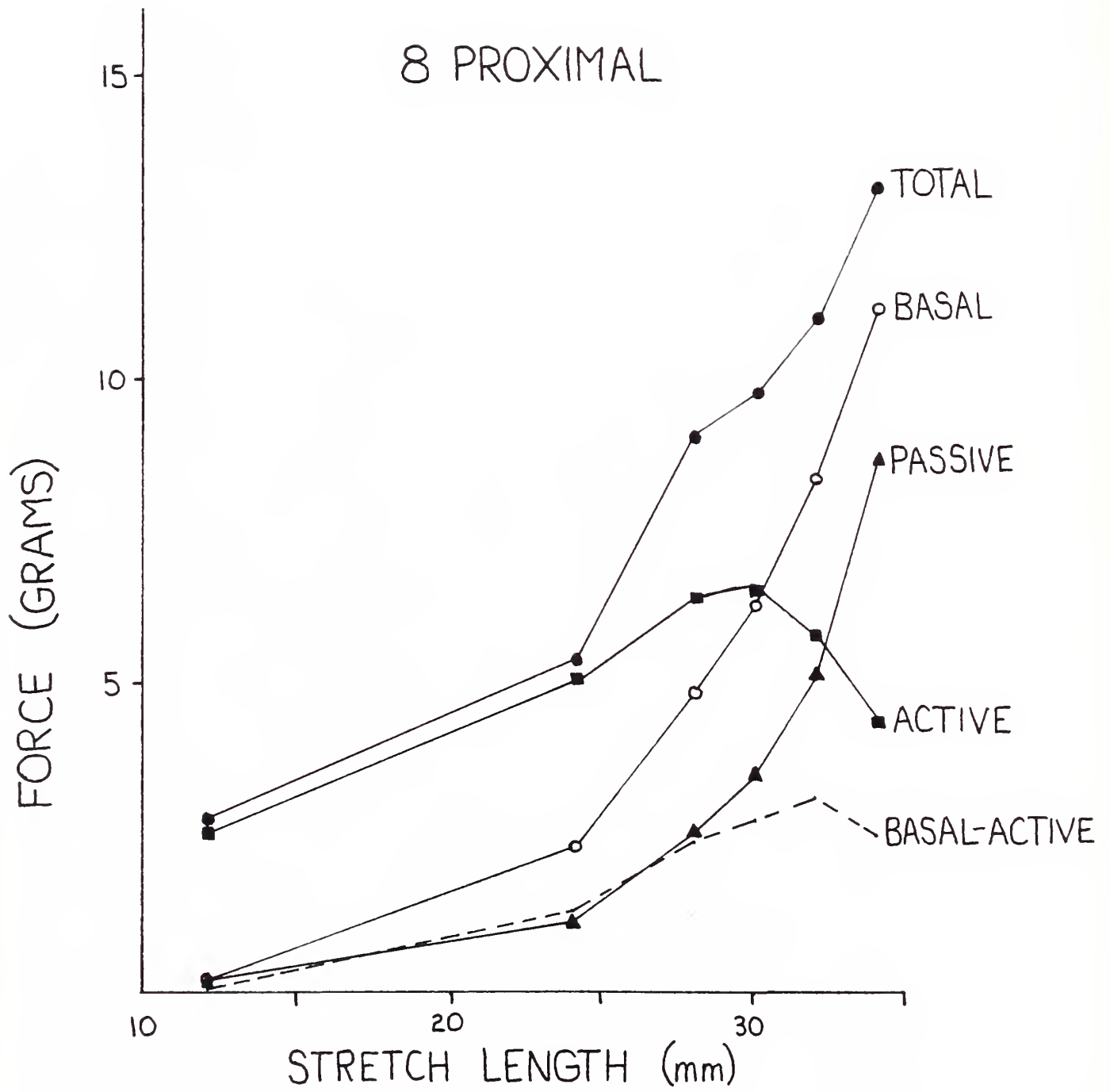








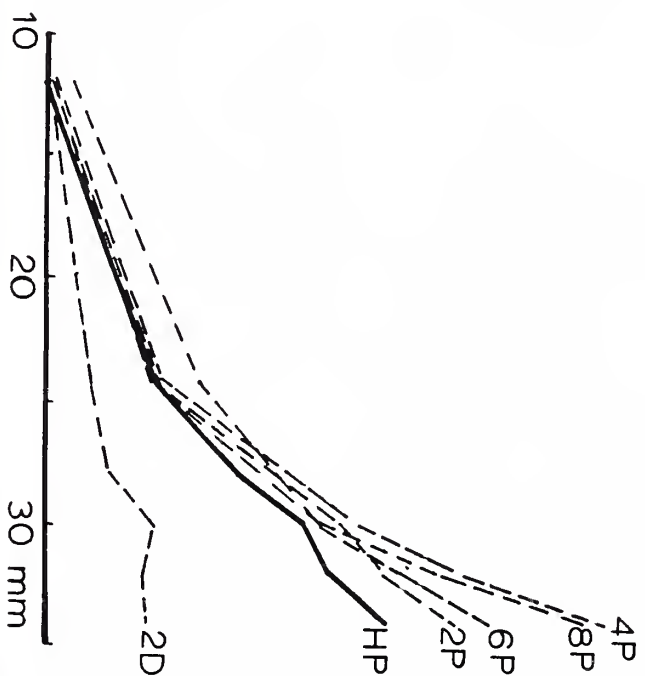
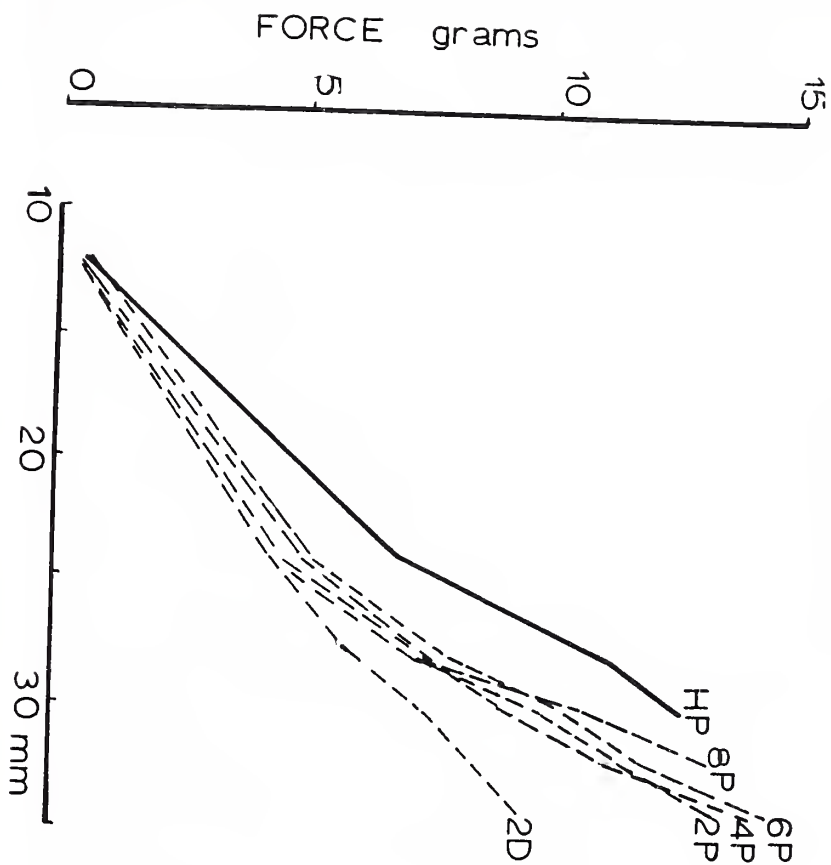




BASAL FORCE

control

esophagitis

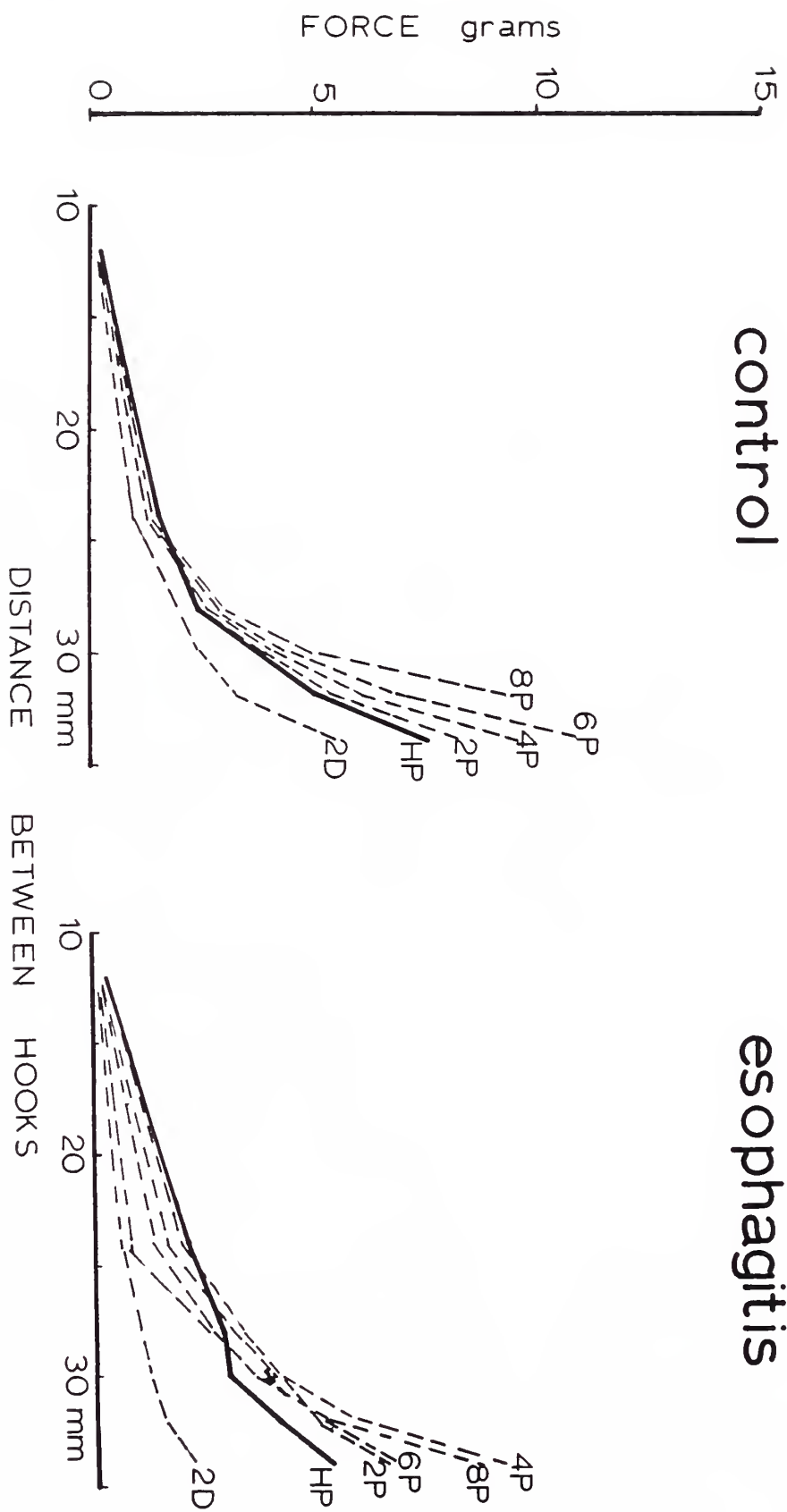


DISTANCE BETWEEN HOOKS

PASSIVE FORCE

control

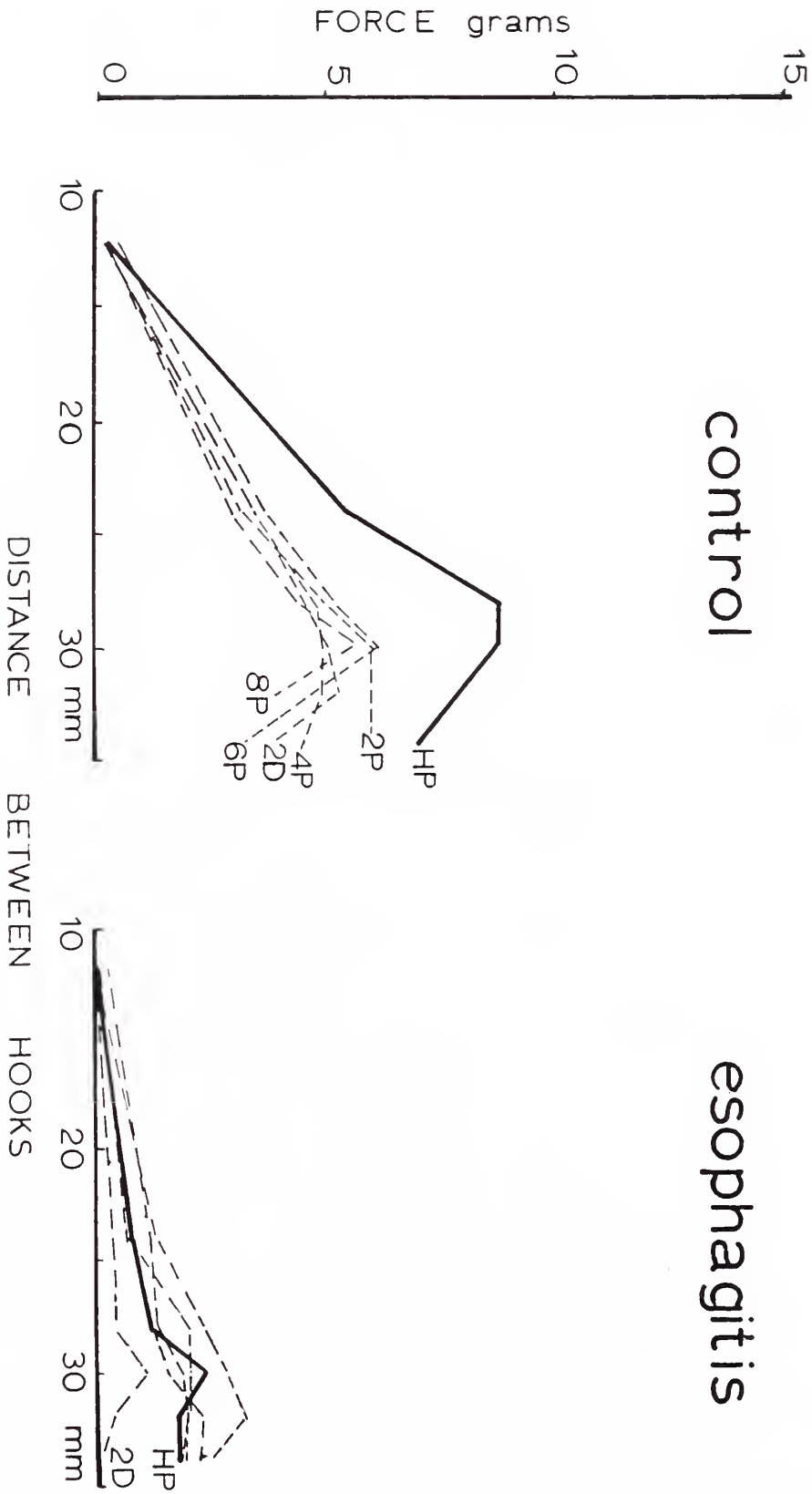
esophagitis



BASAL - ACTIVE FORCE

control

esophagitis

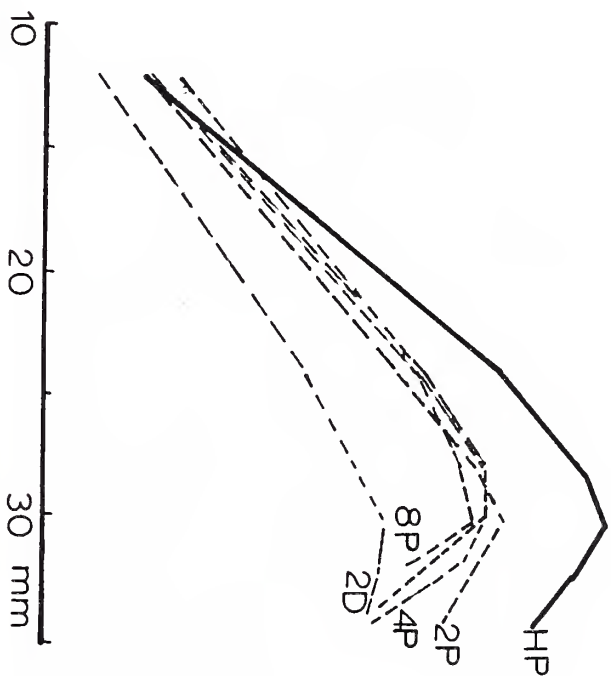


ACTIVE FORCE

control

esophagitis

FORCE grams



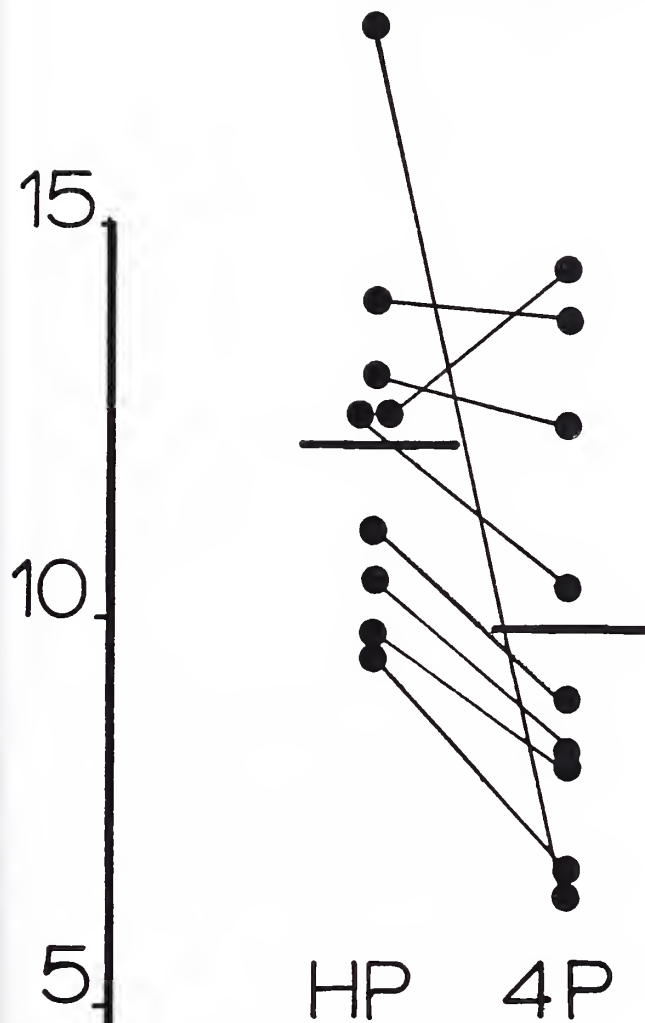
DISTANCE



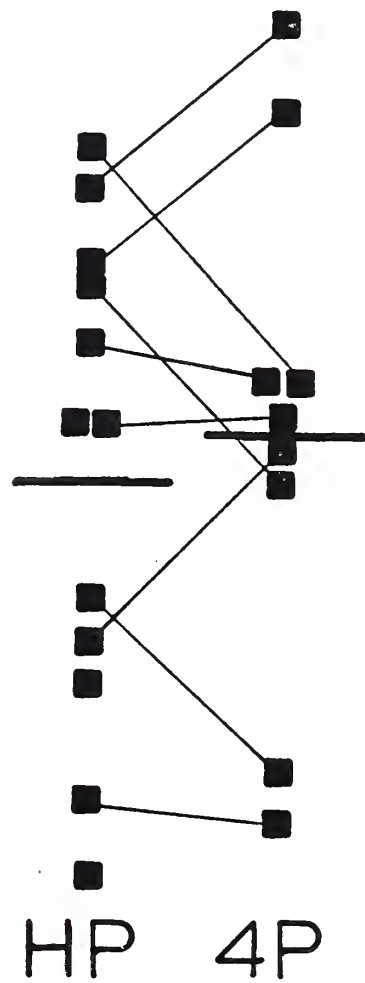
BETWEEN

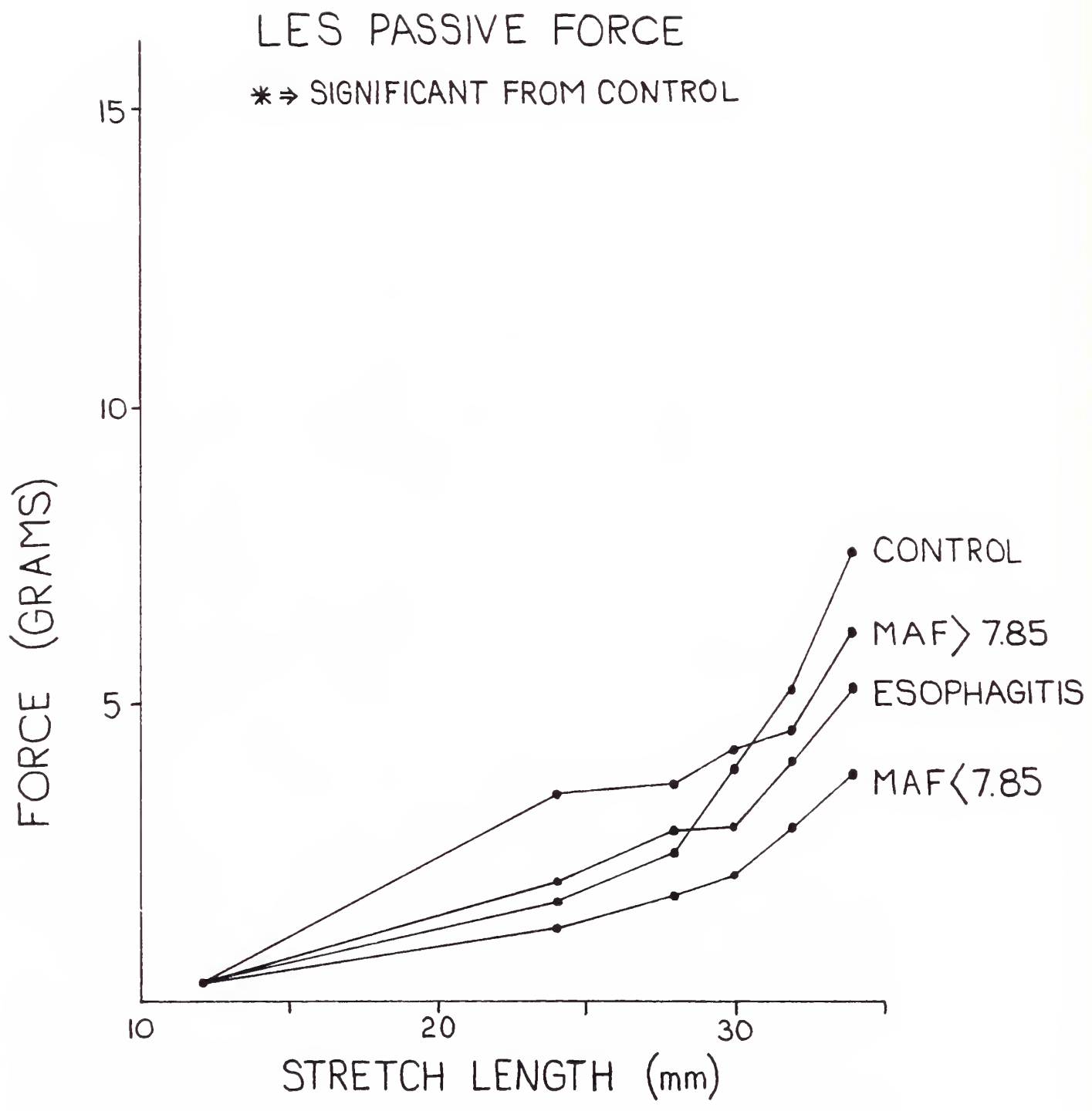
HOOKS

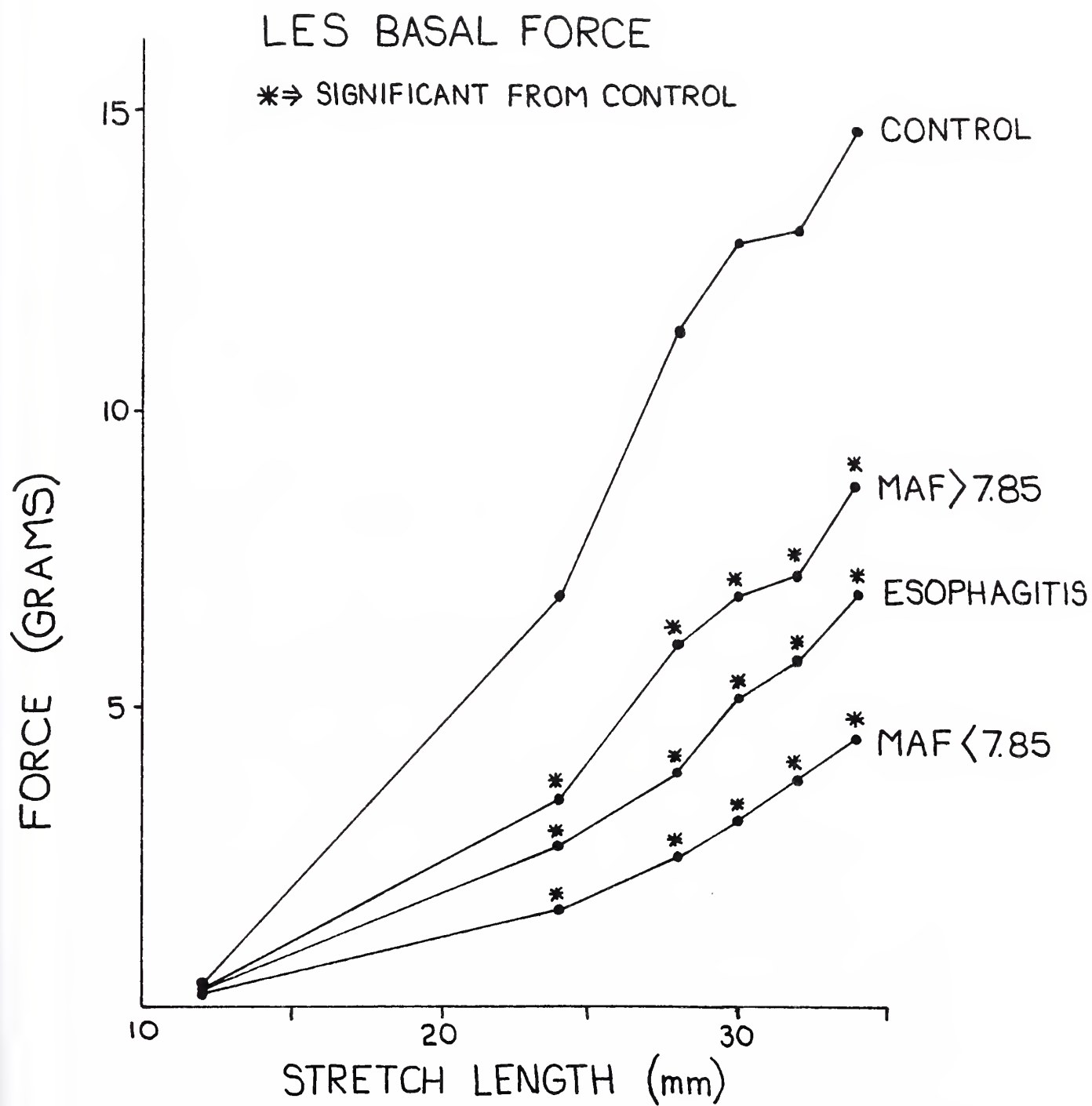
control

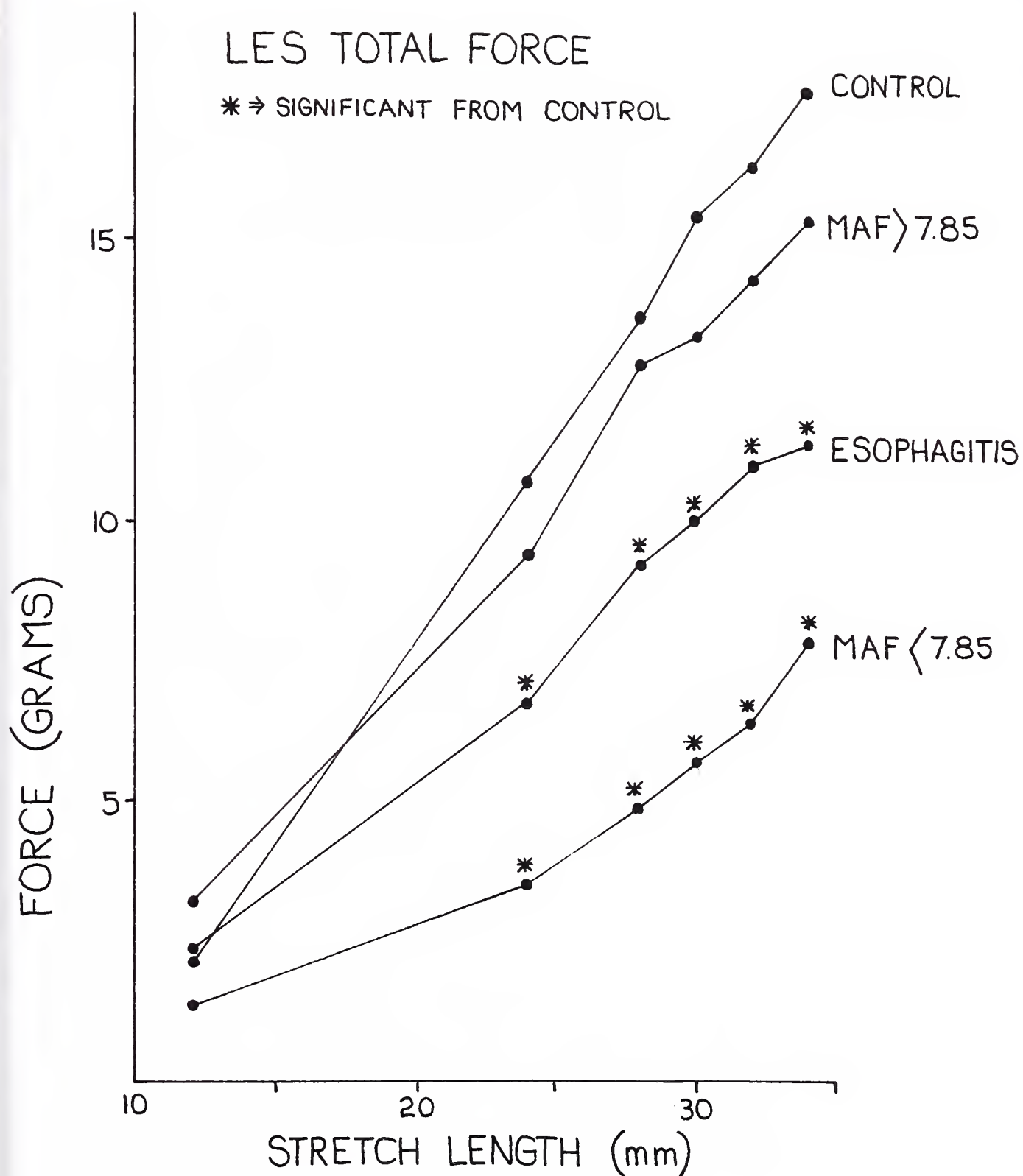


esophagitis



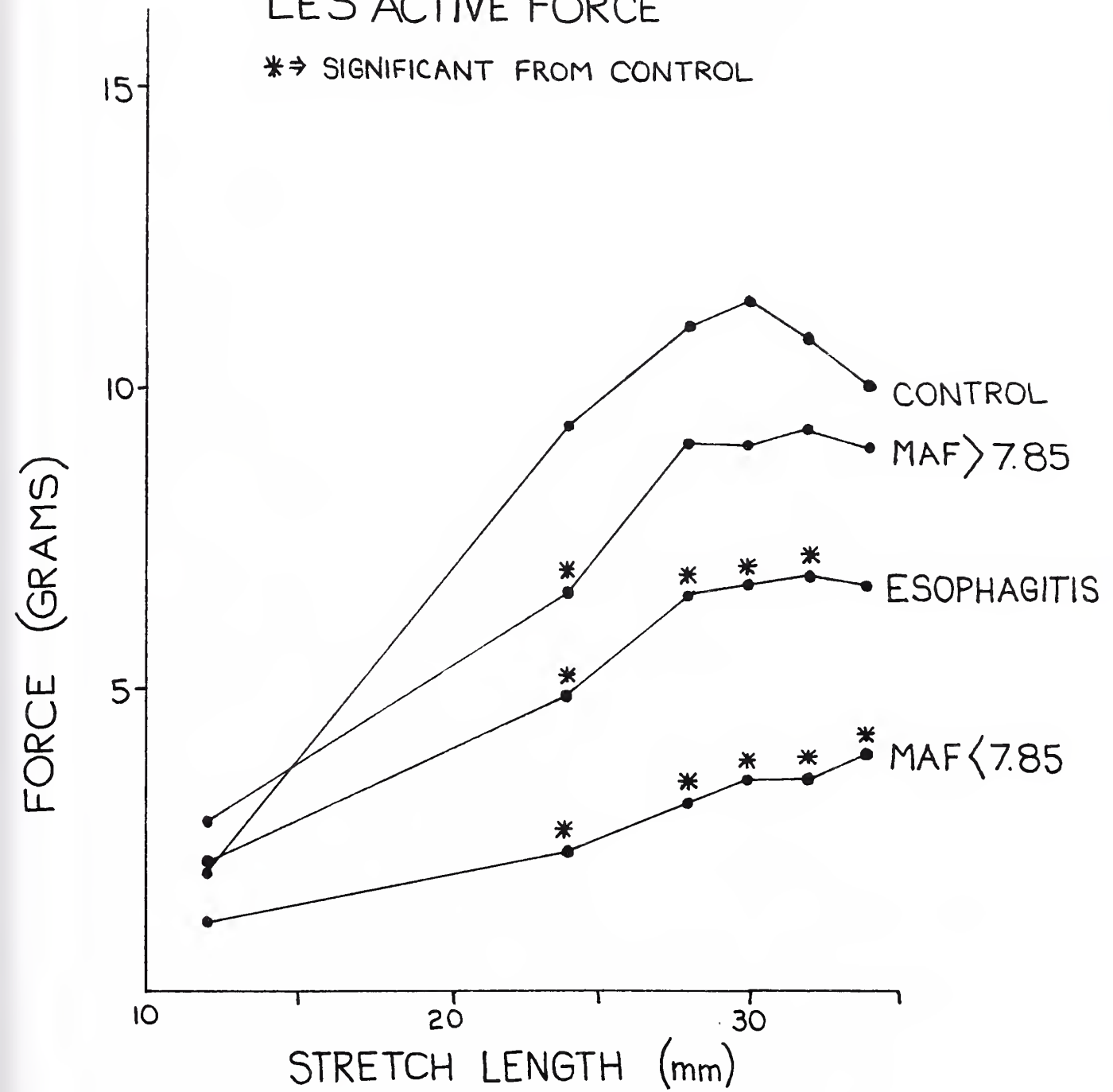






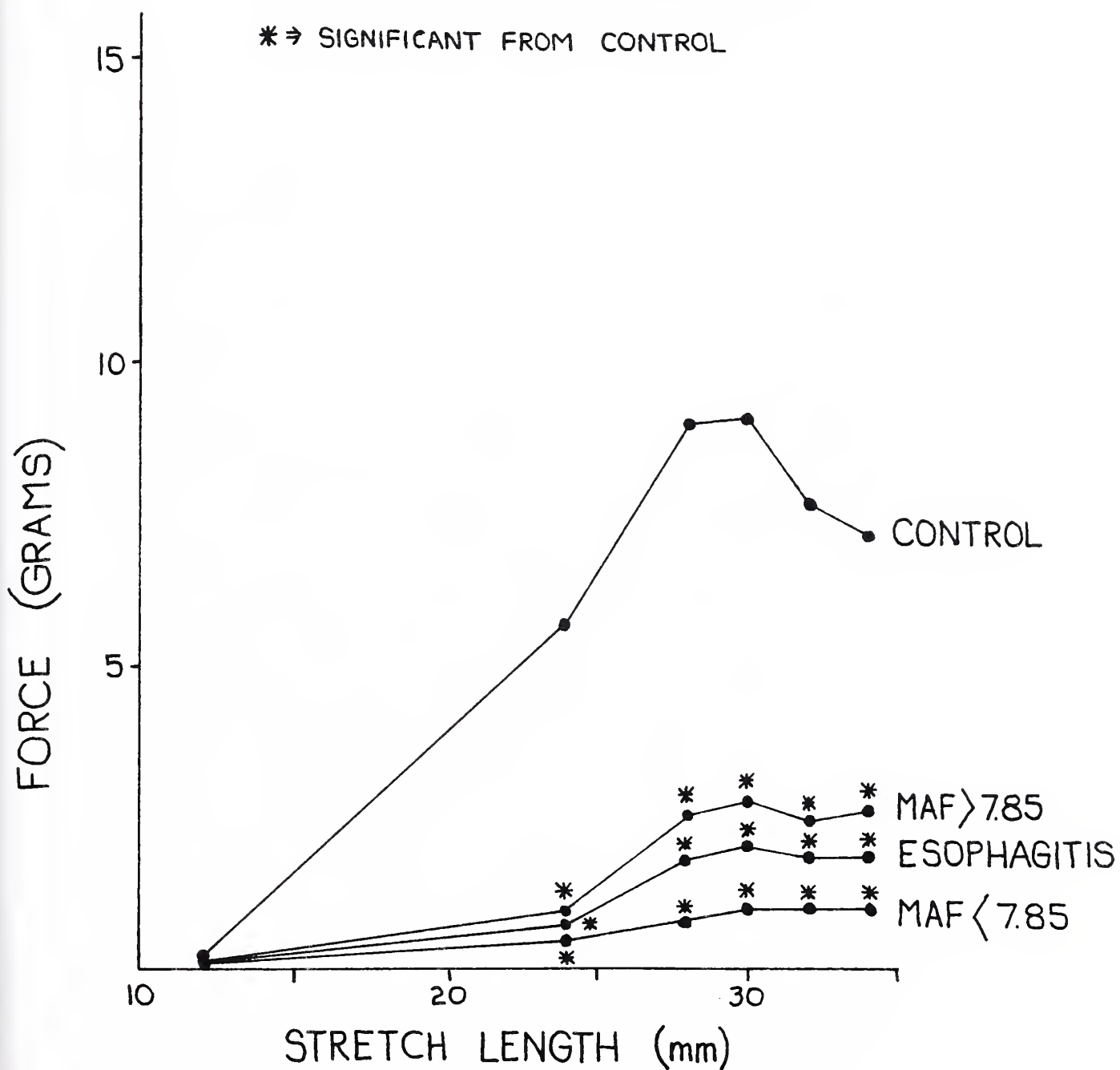
LES ACTIVE FORCE

*⇒ SIGNIFICANT FROM CONTROL



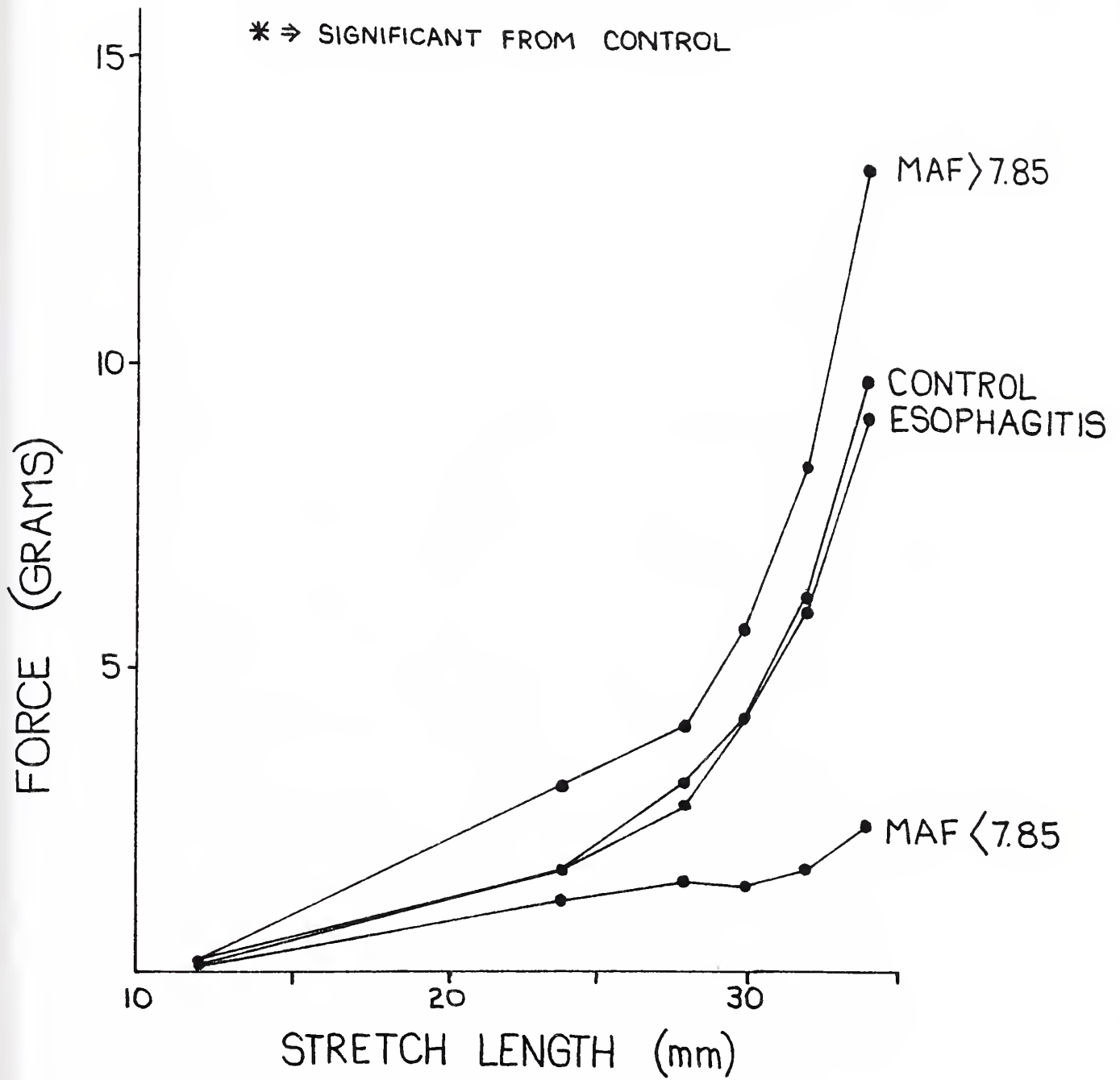
LES BASAL-ACTIVE FORCE

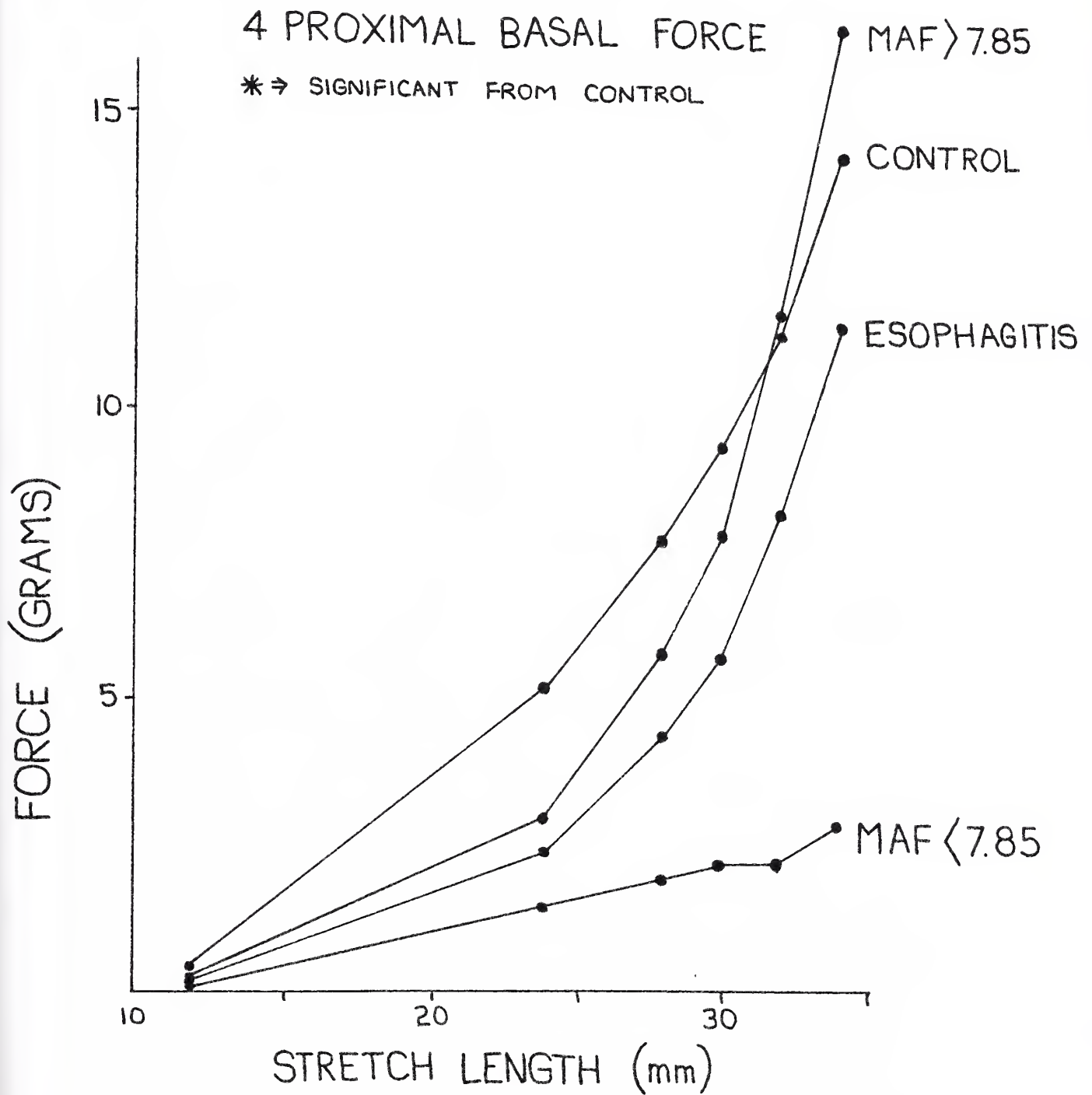
* ⇒ SIGNIFICANT FROM CONTROL

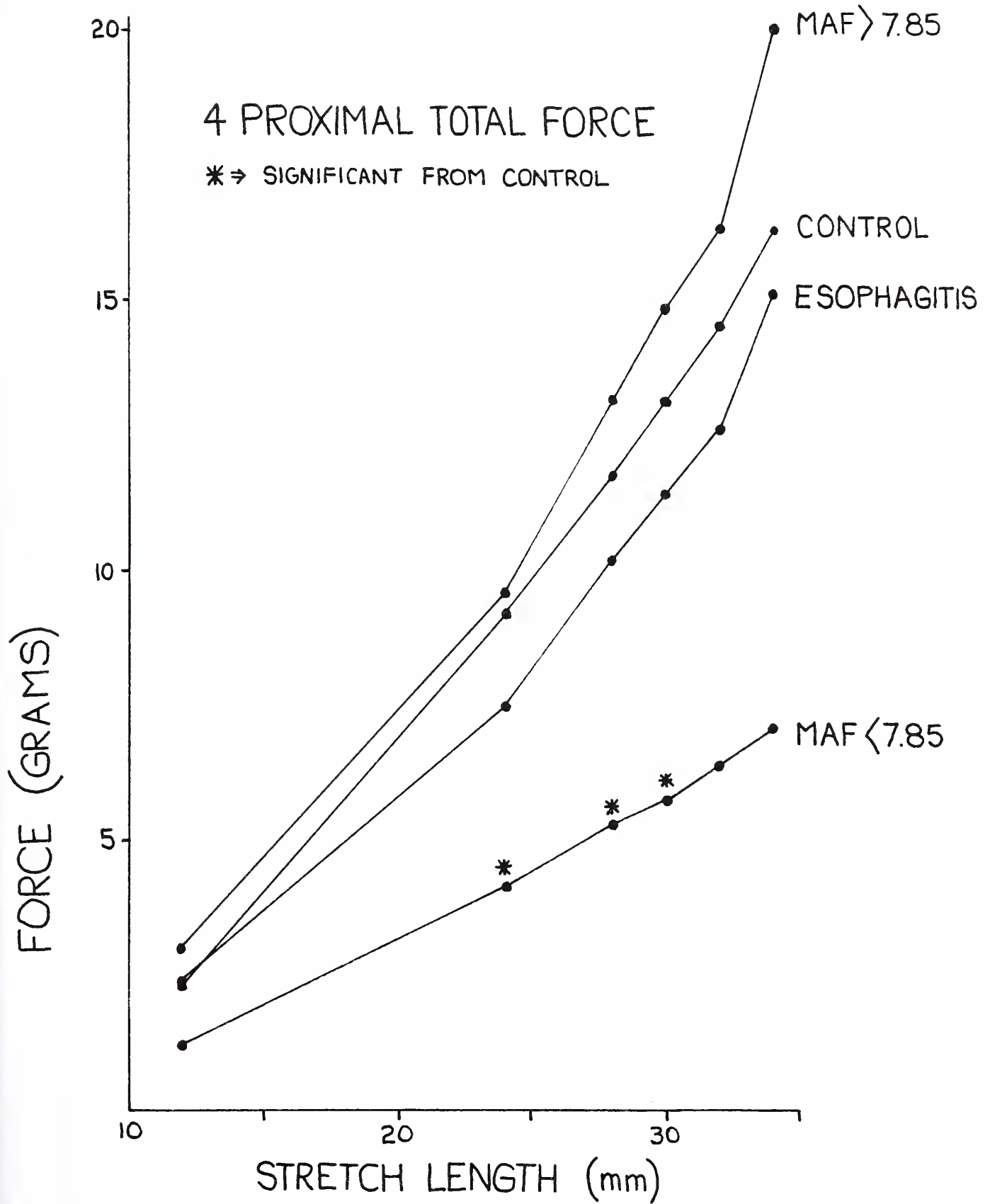


4 PROXIMAL PASSIVE FORCE

* \Rightarrow SIGNIFICANT FROM CONTROL

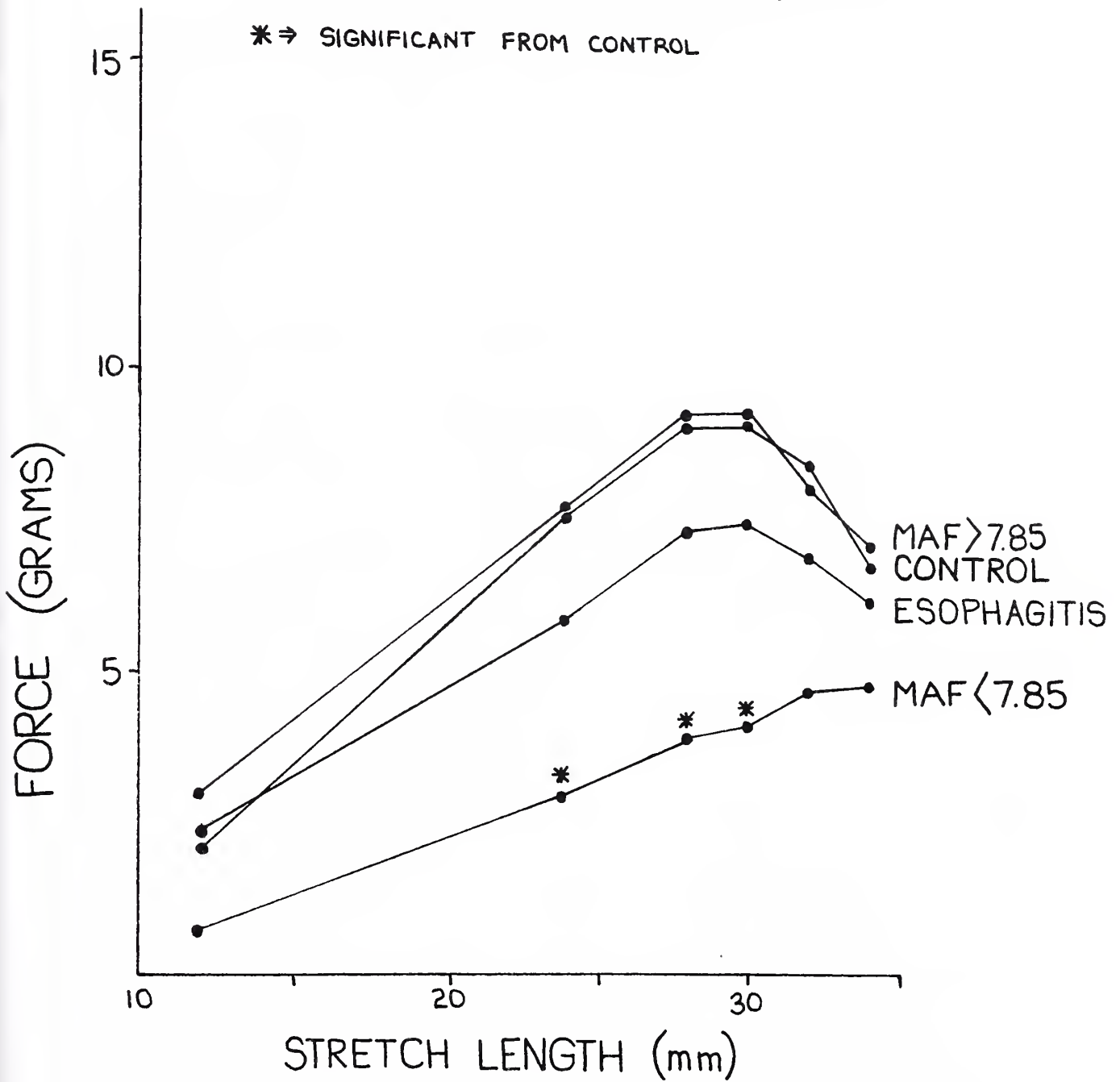






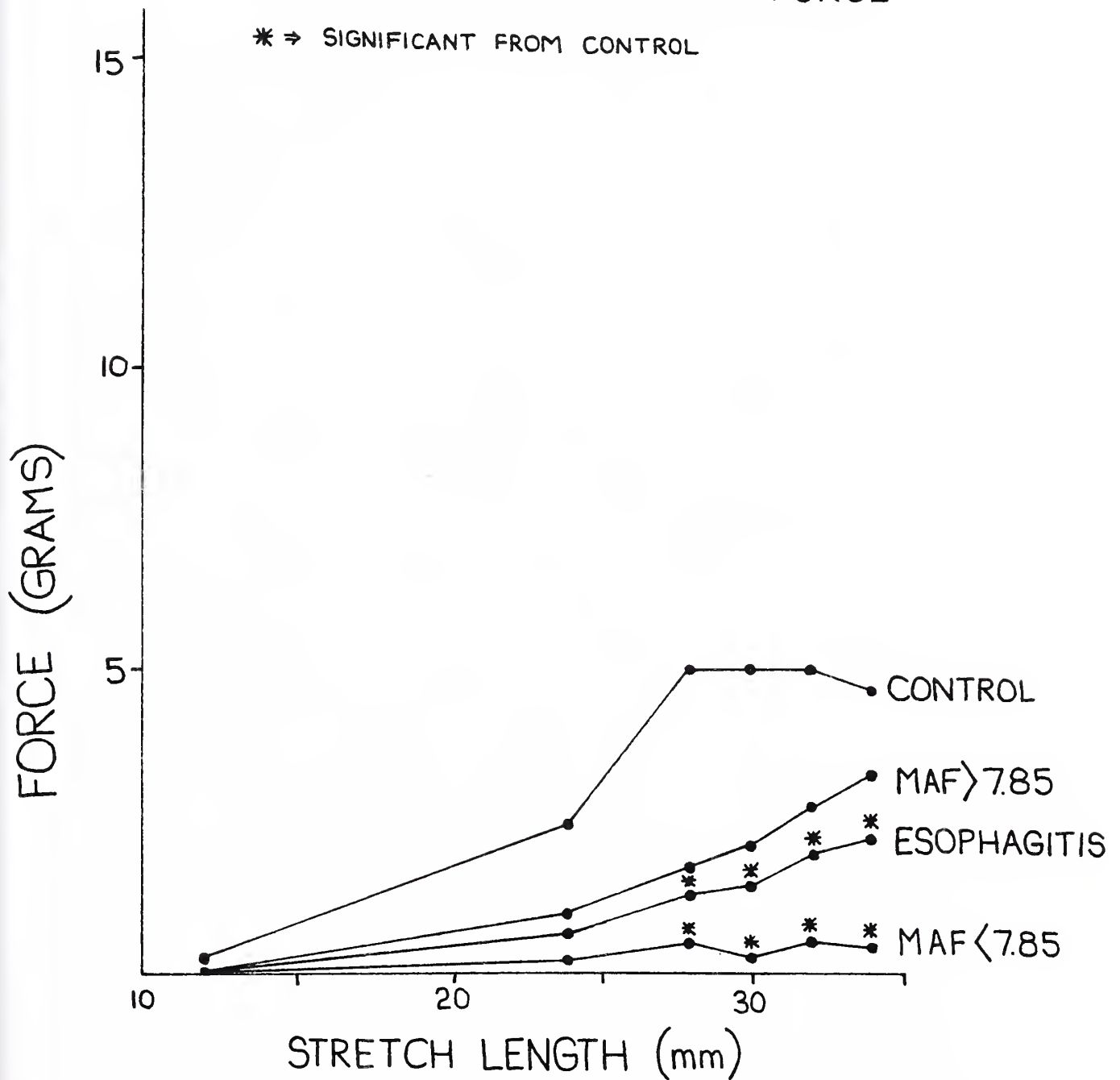
4 PROXIMAL ACTIVE FORCE

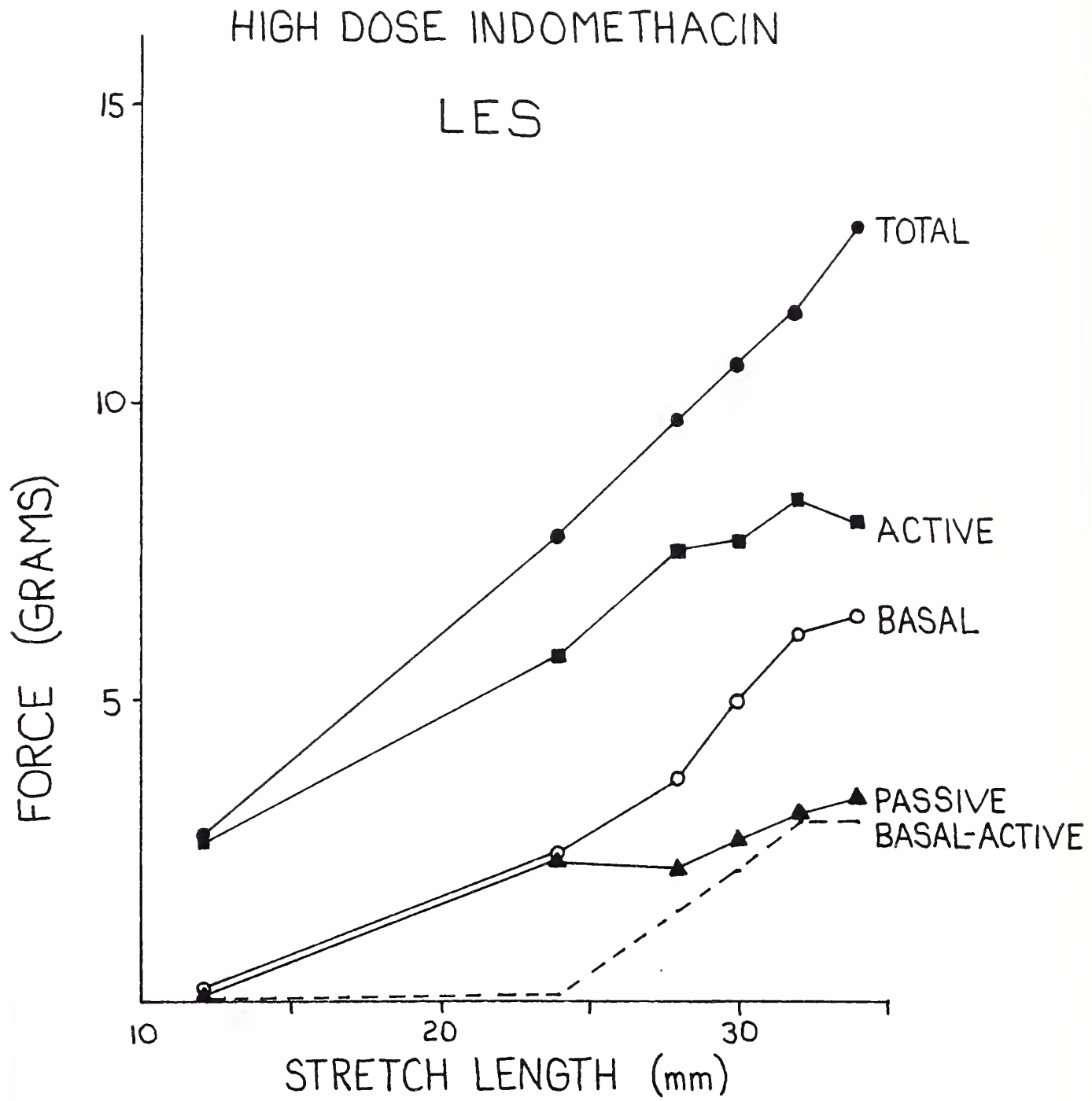
* \Rightarrow SIGNIFICANT FROM CONTROL



4 PROXIMAL BASAL-ACTIVE FORCE

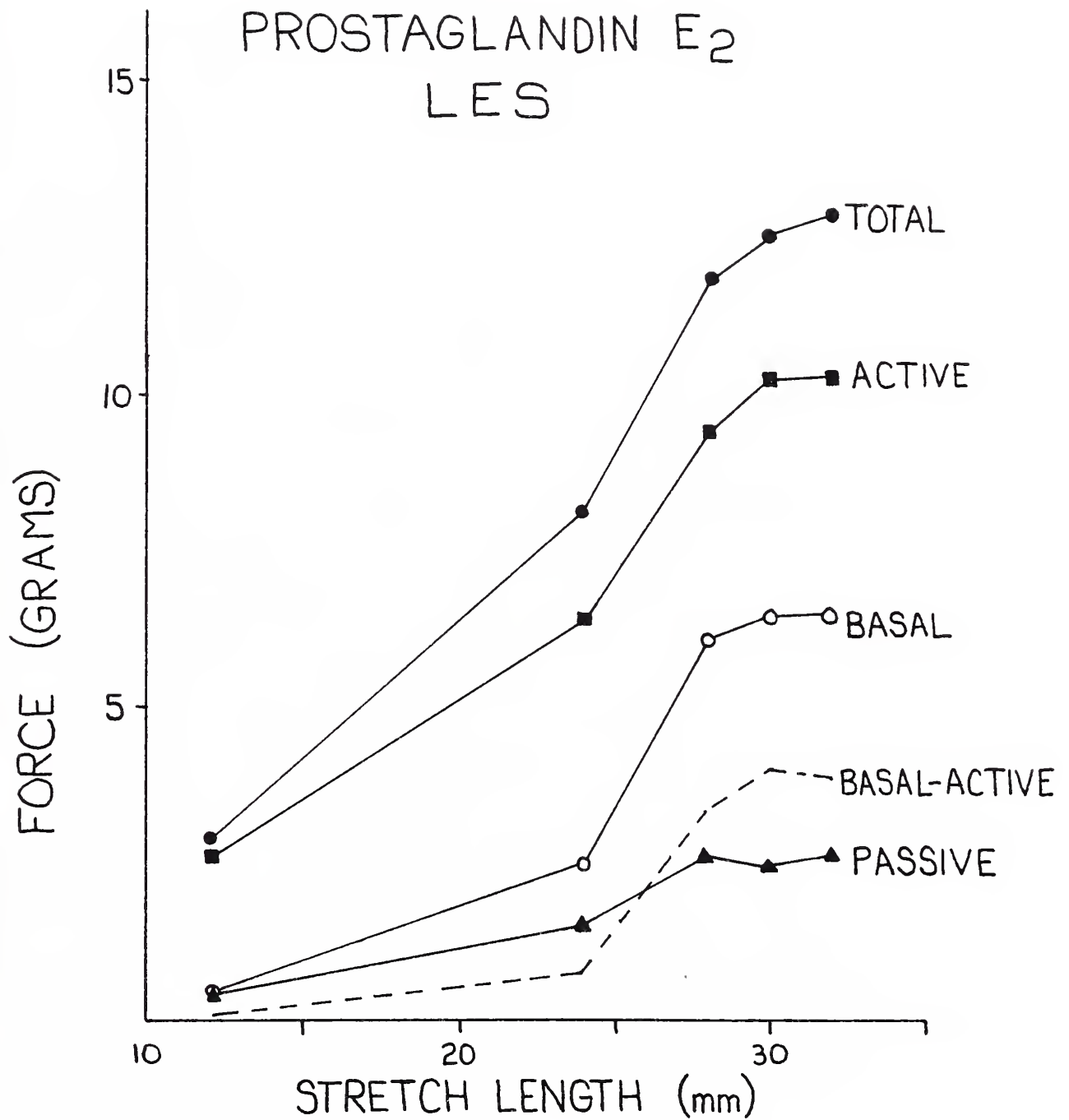
* \Rightarrow SIGNIFICANT FROM CONTROL

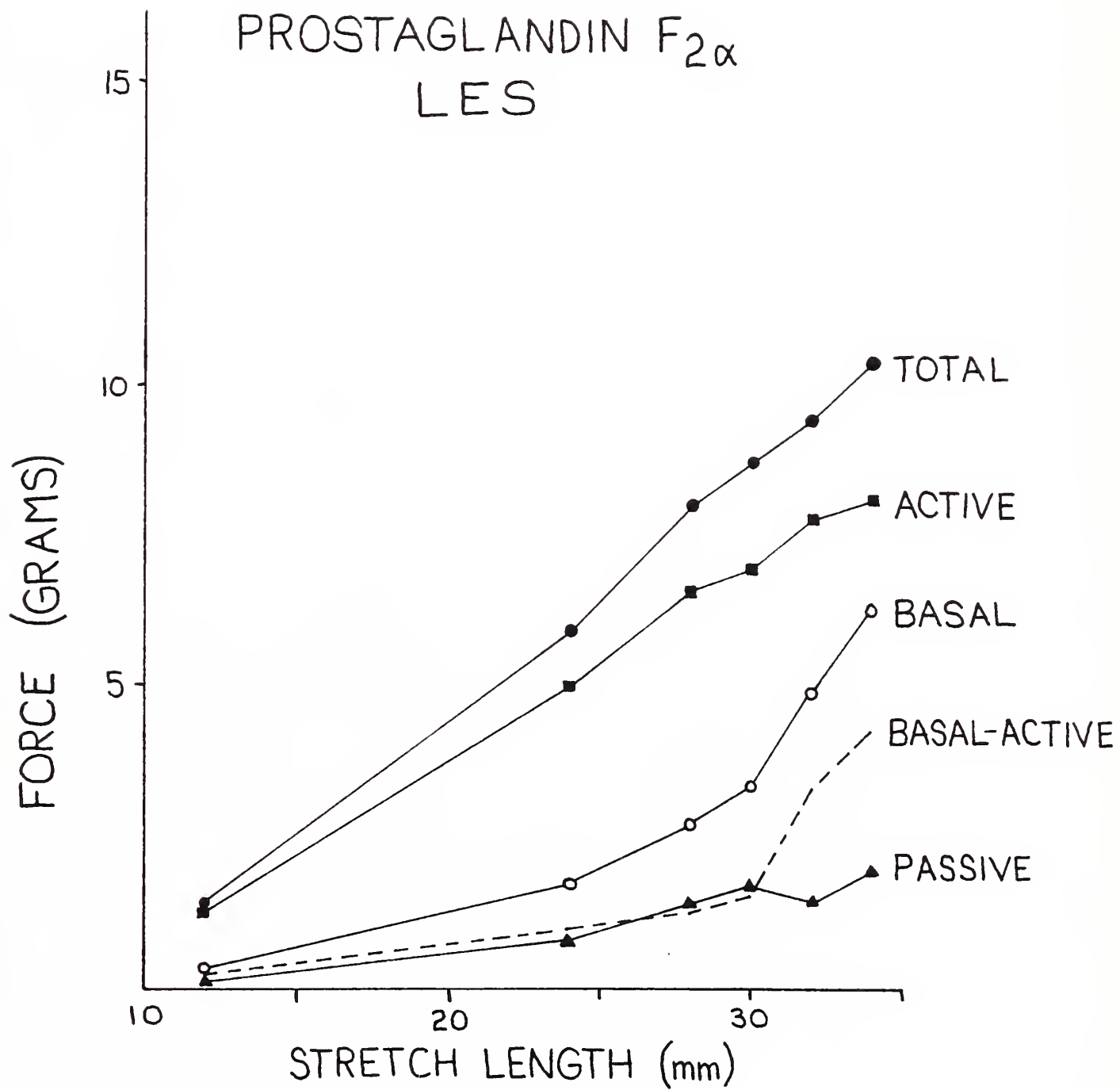


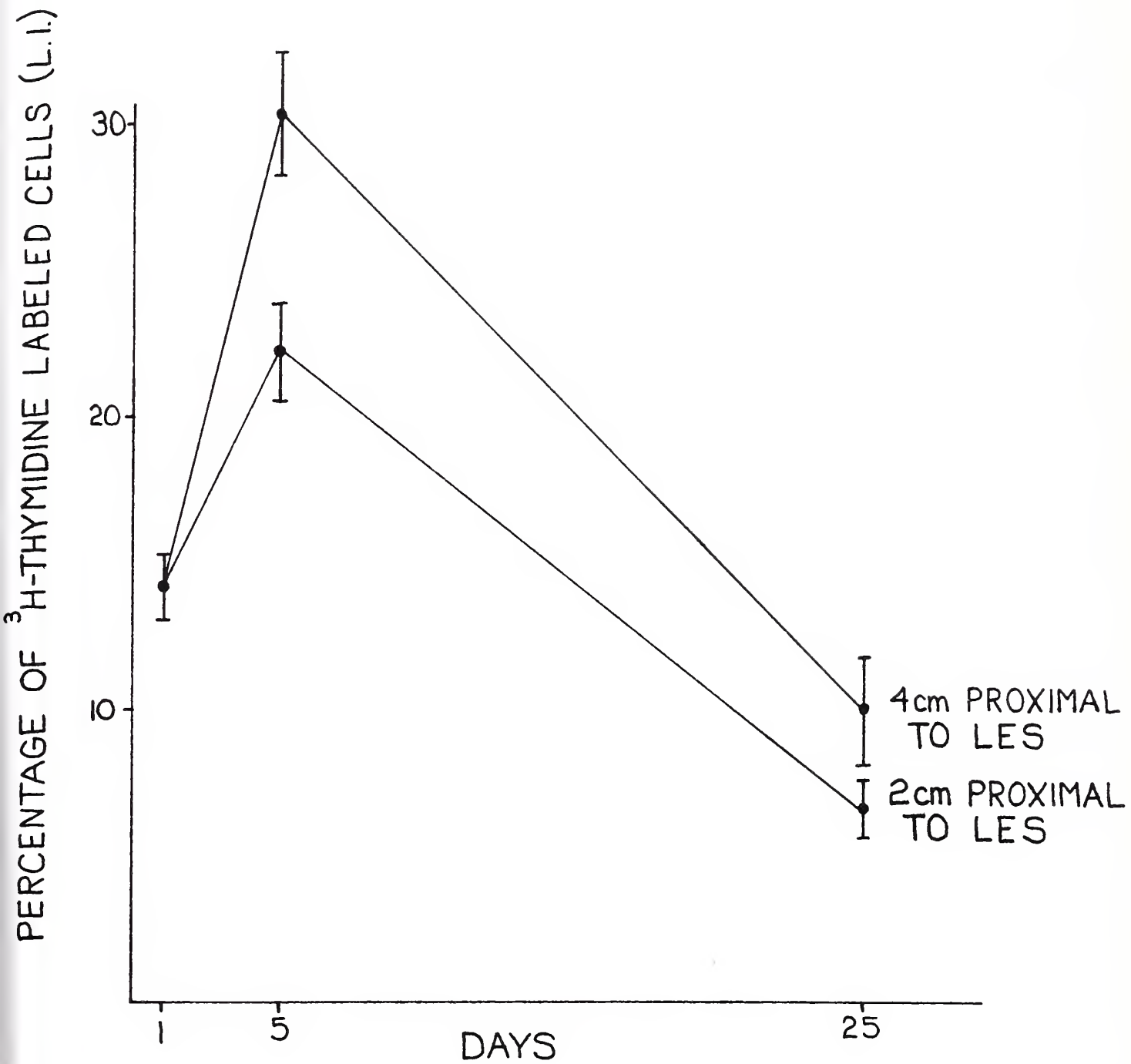


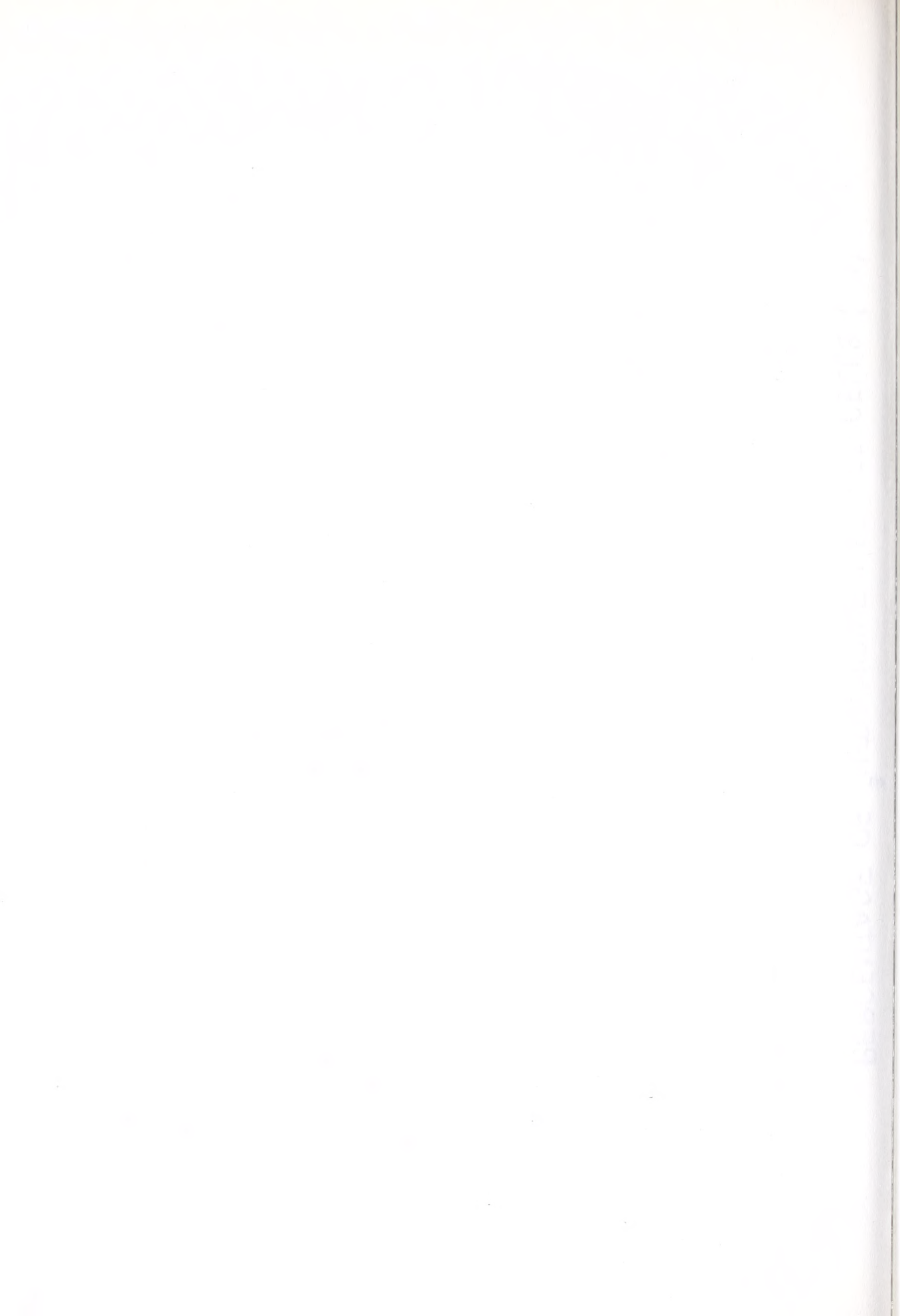
IN VITRO FORCE COMPARISON
AT A STRETCH LENGTH
OF 32 mm
(MEAN \pm SEM GRAMS)

	N	BASAL FORCE	ACTIVE FORCE	TOTAL FORCE
NON-ACID CONTROL	9	12.9 \pm 1.9	10.9 \pm 1.1	16.2 \pm 1.6
ACID CONTROL	12	5.7 \pm 1.2	6.9 \pm 0.9	10.8 \pm 1.5
INDOCIN (4mg/kg)	6	6.1 \pm 1.6	8.4 \pm 0.9	11.5 \pm 2.3









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